Protein A ELISA Kit (9000-1)

For the detection of Natural & Recombinant Protein A

Quick Reference, User Guide and Safety Data Sheets

QUICK REFERENCE GUIDE

Sample Preparation

Select appropriate sample preparation method (see Section 2.2 of User Guide) and prepare samples.

Method	Description
A – Buffer Exchange	Samples are buffer-exchanged into PBS (by dialysis or spin column) then diluted to 0.5 mg/mL in PBS prior to *Test Sample Dilution Prep* (Section 2.4)
B – Dilute & Go	Samples are diluted in PBS 0.1% Tween 20 at least ten-fold, to 0.5 mg/mL, before performing *Test Sample Dilution Prep* (Section 2.4)
C – Boil & Boost	Samples are diluted to ≤15 mg/mL if necessary in neutral buffer. Sample composition is then adjusted to 0.1% Tween 20. Samples are boiled for 5 minutes and centrifuged prior to *Test Sample Dilution Prep* (Section 2.4)

Reagent Preparation

1. Make 1X Sample Diluent: 4.0 mL Reagent A + 16 mL dH_20.
2. Prepare PBS solution: reconstitute 1 PBS pack in 1L water; filter.
3. Make PBS-Tween 20: 700 mL PBS + 700 µL Tween 20; filter.

Microtiter Plate Setup

1. Design experiment and set up microtiter wells as needed.
2. Make sure all wells are level and wash 3x with HPLC or distilled grade water.

Antibody Sample Preparation

1. For each sample: 200 µL Reagent A + 550 µL dH_20 and vortex.

2. Add 250 µL of sample to be assayed (1:4 dilution), vortex, and let stand 5-10 minutes at room temperature.
   Note: For Methods A & B, antibody concentration must be ≤ 0.5 mg/mL.

Protein A Standard Curve

1. Make Protein A Standard solution:
   Tube 1: 10 µL Reagent B + 990 µL 1X Sample Diluent
   Tube 2: 10 µL Tube 1 + 990 µL 1X Sample Diluent
   Tube 3: 16 µL Tube 2 + 984 µL 1X Sample Diluent
   Note: Tube 3 is the 1.6 ng/mL Standard solution.

2. Vortex and let stand 10 minutes at room temperature before sequential serial dilutions.

Preparation of 2-Fold Serial Dilutions of Antibody Sample

These instructions describe preparation of a 7-point standard curve in triplicate. Two-fold dilution series of the standard curve and antibody samples are used starting in wells 1H-3H and 4D-6D respectively.

1. Add 100 µL of 1X Sample Diluent solution into all wells that will contain serial diluted antibody samples or serial diluted Protein A Standard. Do not add Sample Diluent to wells that will contain initial aliquots of antibody sample or Protein A Standard.
2. Transfer 200 µL of prepared 1.6 ng/mL Standard solution (Tube 3) into wells 1H-3H.
3. Transfer 200 µL of the prepared antibody sample into wells 4D-6D.
4. Make 2-fold serial dilutions of the Protein A Standard and antibody sample simultaneously by transferring 100 µL down the plate column.
5. From the final wells containing serial diluted Standard or antibody sample discard 100 µL of solution, leaving 100 µL in each well.

ELISA Testing

1. After serial dilutions, cover the plate and incubate for 30 minutes at room temperature.
2. Allow TMB substrate (Reagent E) to come to room temperature (protect from light.)
3. After 30 minutes, wash plate with PBS-Tween 4 times.
4. Prepare probe: 70 µL Reagent C + 12 mL PBS-Tween.
5. Add 100 µL diluted probe to each well except wells 1A-3A (substrate blanks.)
6. Incubate 30 minutes at room temperature.
7. After 30 minutes, wash 4 times with PBS-Tween.
8. Prepare conjugate: 12 µL Reagent D + 12 mL PBS-Tween.
9. Add 100 µL conjugate solution to each well except wells 1A-3A.
10. Incubate 30 minutes at room temperature.
11. After 30 minutes, wash 2 times with PBS-Tween and then 2 time with PBS only.
12. Add 100 µL TMB substrate to each well and incubate 4 minutes.
13. Stop reaction with 100 µL of 1N Phosphoric acid.
14. Read the plate at 450 nm.

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www.repligen.com

Cut or tear along dotted line

Protein A ELISA Kit (9000-1)

For the detection of Natural and Recombinant Protein A

User Guide and Safety Data Sheets

Note: For safety information in additional languages, please refer to our website at: www.repligen.com/resources/quality-documents/

USER GUIDE

Information contained in this document is subject to change without notice.

This ELISA kit is intended FOR RESEARCH USE ONLY. It is not intended for use as a diagnostic in humans or animals. Repligen Corporation makes no warranty of any kind for this material including, but not limited to, the implied warranties of merchantability and fitness for use. Repligen Corporation shall not be liable for errors contained herein or for incidental or consequential damages related to the furnishing, performance, or use of this material.

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For questions see Section 4, “Troubleshooting,” on page 11 of this User Guide. For further information, please contact Repligen Corporation at www.repligen.com.

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1. Overview of ELISA

Repligen's Protein A ELISA kit (P/N 9000-1) provides accurate, precise and linear quantitation of residual Protein A. Our Protein A detection ELISA kit has been developed for those customers who require a reliable, easy-to-use, highly-sensitive assay to measure small amounts of Protein A in therapeutic protein products.

Testing for residual Protein A occurs in several different phases of development and commercial manufacturing that may include:

- Process development: leaching characteristics of the resin under specific conditions
- Manufacturing: eluted samples taken throughout several points in the purification process
- Finish product release: document process containment levels and lot-to-lot consistency

The kit is supplied with Repligen's recombinant Protein A standard (rProtein A™), one of several Protein A ligands used in affinity chromatography for the purification of monoclonal antibodies. The Repligen Protein A ELISA kit may also be used to quantitate other Protein A variants following appropriate procedures, including but not limited to natural Staphylococcus aureus Protein A and other forms of recombinant Protein A. Repligen also offers a separate Protein A ELISA kit for the detection of MabSelect SuRe™ ligand (part number 9333-1).

The polystyrene microtiter plate provided in this kit is coated with anti-Protein A antibodies. rProtein A™ Standards and Test Samples are diluted with sample diluent (Reagent A) and incubated with the immobilized antibodies. Captured rProtein A™ is then detected by the addition of Biotinylated anti-Protein A probe (Reagent C). The high substitution of the probe allows maximum binding of Streptavidin Peroxidase conjugate (Reagent D). The final detection step involves adding Tetramethylbenzidine, TMB (Reagent E), to give a highly sensitive colorimetric reaction. The color intensity is proportional to the amount of rProtein A™ present in the sample.

1.1 Important points regarding Assay Sensitivity

1. Numerical results of this assay are expressed as nanograms per milliliter (ng/mL) of rProtein A™.
2. The sensitivity of the assay is typically 0.1 ng/mL, which corresponds to a sensitivity or limit of quantitation of 0.8 ppm Protein A per antibody using the Buffer Exchange or Dilute & Go method.
3. The sensitivity of the assay is typically 0.1 ng/mL, which corresponds to a sensitivity of 0.032 ppm using the Boil & Boost method.
4. Assay characterization recommendations are available in Repligen Technical Notes. Please contact Customer Service for a copy or go to www.repligen.com.

1.2 Reagents

Table 1.1 Reagents provided

Reagent	Description	Volume	Storage
Reagent A	Sample Diluent (5X) concentrate	20 mL	2-8°C
Reagent B	rProtein A™ Standard solution, contains 1.0 mg/mL in sterile water	200 µL	-20°C
Reagent C	Rabbit anti-Protein A: Biotin probe, contains 0.02% sodium azide	200 µL	2-8°C
Reagent D	Streptavidin-HRP (Horseradish Peroxidase) conjugate	200 µL	2-8°C
Reagent E	TMB Peroxidase substrate, contains 3, 3', 5, 5'-tetramethylbenzidine in buffer	20 mL	2-8°C
PBS Packs	Final volume of each pack when reconstituted is 1 L	2 packs	Ambient
ELISA Plate	96-well microtiter plate coated with anti-Protein A, packed with desiccants	Dried Plate	2-8°C

Note: Reagents are specific to the kit lot and should be discarded once all plate strips have been consumed.

Table 1.2 Reagents, supplies, and equipment not provided with the kit

dH_2O or HPLC-Grade water (preferred)	Filter (0.22 µm) and 1 L bottle
1 L graduated cylinder	Phosphoric acid
1.5 mL Eppendorf tubes	Micro-pipettors and 12-channel pipettor
15 and 50 mL plastic centrifuge tubes	ELISA plate reader with wavelength capability at 450 nm
Tween 20	Timer
Reagent reservoirs	Vortex mixer
5 and 10 mL Serological pipettes	Micro-centrifuge
Plate sealers	Water bath

2. Guide to Standard Preparation and Assay

2.1 Pre-Assay Reagent Preparation

All Kit Components

Allow all kit components to equilibrate to *room temperature, including the frozen Reagent B.

1X Sample Diluent

Dilute 4.0 mL of Reagent A (5X Sample Diluent) in 16 mL of purified water in a 50 mL plastic centrifuge tube. Vortex for 5-20 seconds or invert 10-15 times for thorough mixing. If required, the 1X Sample Diluent is stable for 2 weeks at *room temperature.

PBS Solution

Dissolve the contents of one PBS pack in 800 mL of dH_20 to a final volume of 1L. Mix well. Filter PBS solution through a 0.22 µm filter.

PBS-Tween 20 Wash Solution

Pour 700 mL of the PBS solution (prepared & filtered per instructions above) into a 1L graduated cylinder. Add 700 µL of Tween 20. Mix well. Save the remaining 300 mL PBS solution for the final ELISA wash. Filter PBS-Tween solution through a 0.22 µm filter.

TMB Substrate Solution

For a full-plate assay, use the whole bottle of TMB. For half-plate assays, aliquot 8 mL of TMB into a
15 mL conical centrifuge tube and cover with aluminum foil. Return bottle to the 2-8°C refrigerator.

Test Samples – Allow all test samples to equilibrate to *room temperature.
10% Tween 20 – Prepare a 10% Tween 20 solution only if using the Boil & Boost method.

***Note: An ideal room temperature range of 60-72°F (16-22°C) is important for optimum assay performance**.

2.2 Sample Preparation Methods

Sample preparation methods for the Protein A ELISA assay have been optimized to allow end users to select the method most appropriate for their assay needs. A representative preparation method overview is shown in Table 2.1.

Table 2.1 Preparation Method Overview (with starting concentrations)

Desired LOQ	Input Sample Conc. Constraint	Method	Description
~ 0.8 ng/mg	N/A	A – Buffer Exchange	Samples are buffer-exchanged into PBS (by dialysis or spin column) then diluted to 0.5 mg/mL in PBS prior to *Test Sample Dilution Prep* (Section 2.4)
~ 0.8 ng/mg	≥ 5.0 mg/mL antibody	B – Dilute & Go	Samples are diluted in PBS 0.1% Tween 20 at least ten-fold, to 0.5 mg/mL, before performing *Test Sample Dilution Prep* (Section 2.4)
~ 0.03 ng/mg	≤ 15mg/mL of antibody	C – Boil & Boost	Samples are diluted to ≤ 15 mg/mL if necessary in neutral buffer. Sample composition is then adjusted to 0.1% Tween 20. Samples are boiled for 5 minutes and centrifuged prior to *Test Sample Dilution Prep* (Section 2.4)

Table 2.2 Method Attribute Table

	A: Buffer Exchange	B: Dilute & Go	C: Boil & Boost
High Performance	X	X	X
Assay completion < 2 hours	X	X	X
Reduced Sample Preparation Steps		X	
Enhanced Limit of Quantitation			X
High starting sample concentration			X

Method A: Buffer Exchange

Prior to running the assay, samples must be buffer-exchanged into PBS (0.01 M phosphate buffer, 0.15 M sodium chloride, 0.003 M potassium chloride, pH 7.2-7.4) and diluted to a protein concentration of ≤ 0.5 mg/mL. Dialysis or a desalting column may be used.

Note: The PBS packs provided in the kit are not intended for this buffer exchange. They are to be reconstituted and used as directed in the ELISA protocol.

Method B: Dilute & Go

This method is designed to dilute out any interfering substances. It can perform with common process buffers such as 100 mM Citrate, Glycine, and Acetic buffers neutralized with Tris-base. Prior to running the assay dilute Protein A-purified antibody samples, with starting concentrations greater than 5.0 mg/mL, directly into phosphate buffered saline (PBS) with 0.1% Tween 20 to reach a final concentration of 0.5 mg/mL. For best performance, characterize the assay with process-specific buffers and proteins.

Note: No buffer exchange is required when the dilution step is performed. If sample concentration is less than 5.0 mg/mL, the Dilute & Go method (Method B) is not recommended. Instead, the user should proceed with Buffer Exchange (Method A).

Method C: Boil & Boost

This method is designed for high input antibody concentrations. It has been shown to perform with common process buffers such as 100 mM Citrate and Acetate neutralized with Tris-base at antibody concentrations up to 15 mg/mL. Characterize the assay with process-specific buffers and proteins for performance.

Note: Protein A recovery in Glycine buffers or with > 0.2% Polysorbates was observed to be significantly lower than other buffers when this method was used. It is recommended that samples containing Glycine or high concentrations of surfactants be buffer-exchanged into PBS prior to running this method.

Add at least 0.5 mL of each sample to 1.5 mL centrifuge tubes (the assay procedure will require 0.25 mL.) Tween 20 should be added to each sample to a final concentration of 0.1%. Create a pin hole in the cap of each centrifuge tube and boil for 5 minutes in a water bath. After cooling the samples, centrifuge the tubes at 13,000 x g or 1,000 rpm for 5 minutes. Boiling causes disassociation from Protein A and precipitation of IgG. Transfer the supernatant to a new tube (optional). The supernatant will be used when preparing sample dilutions in the assay procedure.

2.3 Standard Preparation

1. When Reagent B is completely thawed, mix by vortex. If reagent remains on the sides or cap of the tube, briefly spin in a micro-centrifuge.
2. Label three 1.5 mL Eppendorf tubes as Tube 1, Tube 2, and Tube 3. Prepare the most concentrated standard solution (1.6 ng/mL rProtein A™, Tube 3) by diluting Reagent B with 1X Sample Diluent per Table 2.3 below. (Vortex each tube thoroughly between dilutions).

Table 2.3 Concentrated Standard Solution Preparation

Tube	Protein A Standard	1X Sample Diluent
1	10 µL of Reagent B	990 µL
2	10 µL of Tube 1	990 µL
3	16 µL of Tube 2	984 µL

3. Place Tube 3 (1.6 ng/mL Protein A Standard) off to the side.

2.4 Test Sample Dilution Preparation

1. After test samples have been prepared and are at the appropriate starting concentration (per Table 2.1), label an Eppendorf tube for each Test Sample. Add 200 µL of 5X Sample Diluent (Reagent A) to each. Next add 550 µL of dH_2O to each of these tubes. Vortex for
   5-10 seconds to ensure thorough mixing.
2. Test Samples should be fully equilibrated to room temperature before diluting. Add 250 µL of each Test Sample to the labeled tubes. Vortex for 5-10 seconds to ensure thorough mixing. These are the first 1:4 starting sample dilutions. Place these tubes off to the side with the 1.6 ng/mL Standard dilution (Tube 3).
3. Let each Test Sample and the 1.6 ng/mL Standard dilution sit for 10 minutes before pipetting into the assay plate.
4. During the 10-minute incubation, wash the plate three times. Fill the wells with dH_2O by using a wash bottle or automated plate-washing system. Remove the liquid from the plate and repeat. After the third wash, dry the plate by inverting it on clan paper towels and tapping gently.

2.5 Plate Set-up

Note: The following pipetting and suggested dilution instructions are specific to a single sample assay, as shown in Figure 2.1. Analogous steps should be taken when performing the assay according to your personal design. Alternatively, users may choose to prepare standards and samples in a dilution plate and transfer to assay plate.

1. Using a 12-channel pipettor, add 100 µL of 1X Sample Diluent into columns 1-3 rows B-G and columns 4-6 rows A-C.
2. Transfer 200 µL of 1.6 ng/mL Protein A Standard solution (Tube 3) into wells 1H-3H.
3. Transfer 200 µL of 1:4 Antibody Sample dilution into wells 4D-6D
4. Make 2-fold serial dilutions of the Protein A Standard and Antibody Samples by transferring 100 µL from each set of triplicate wells into the well directly above them. Mix thoroughly by pipetting 5 times.
   Note: In a single sample assay format, the same tips can be used for each row.
5. After making the last Protein A Standard serial dilution in wells 1C-3C, remove 100 µL and discard. Also discard 100 µL from the final Antibody Sample dilution in wells 4A-6A.

Figure 2.1 Representative Plate Setup for One Antibody Sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	Plate Blank			1:32								
B	0			1:16								
C	0.05			1:08								
D	0.1			Sample #1, 1:4								
E	0.2											
F	0.4											
G	0.8											
H	1.6											

2.6 ELISA Procedure

1. After the Protein A Standards and Antibody sample dilutions have been prepared, cover the plate and incubate at room temperature for 30 minutes.
2. After incubation, remove all liquid from the wells. Using a wash bottle or automated plate-washing system, wash the plate with PBS-Tween 20 solution. Remove the liquid and dry thoroughly by inverting the plate on clean paper towels and tapping gently. Repeat the wash and dry cycle three more times for a total of four washes.
3. Briefly vortex the Reagent C vial. If reagent material remains on the sides or cap of the tube, briefly spin in a micro-centrifuge. Prepare the Rabbit anti-Protein A Biotin probe solution. For a full plate assay, prepare 12 mL by combining 70 µL of Reagent C with 12 mL of prepared PBS-Tween 20 in a 15 mL conical centrifuge tube. For a half-plate assay, prepare 6mL by combining 35 µL of Reagent C with 6 mL PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
4. Using a 12-channel pipettor, add 100 µL of the diluted Reagent C probe solution to each well containing a Test Sample or Standard. Leave wells 1A-3A (Plate Blanks) empty.
5. Cover the plate and incubate at room temperature for 30 minutes. After incubation, wash the wells four times with PBS-Tween 20 and remove the liquid. Dry thoroughly by inverting the plate on clean paper towels and tapping gently.
6. Briefly vortex the Reagent D vial. If reagent material remains on the sides or cap of the tube, briefly spin in a micro-centrifuge. For a full-plate assay, prepare 12 mL of Streptavidin
   horseradish Peroxidase conjugate solution by combining 12 µL of Reagent D with 12 mL of
   prepared PBS-Tween 20 in a 15 mL conical centrifuge tube. For a half-plate assay, prepare 6mL by combining 6 µL of Reagent D with 6 mL PBS-Tween 20 in a 15 mL conical centrifuge tube. Mix solution thoroughly.
7. Add 100 µL of the diluted Reagent D conjugate solution to each well containing Test Sample or Standard. Leave wells 1A-3A (Plate Blanks) empty.
8. Cover the plate and incubate at room temperature for 30 minutes.

9. After incubation, discard the conjugate solution from the plate. Wash the wells twice with PBS-Tween 20. Wash twice more but with PBS only. After each wash, discard the liquid by inverting the plate on clean paper towels and tapping gently.
   Note: Before proceeding with the next step, make sure the TMB solution is at room temperature (≥ 72°F or 22°C). If the lab is too warm, move the assay to a cooler location for the development step.
10. Using a multi-channel pipettor, add 100 µL of the TMB substrate to each of the wells, **including** 1A-3A (Plate Blanks).
11. Incubate plate for **4 minutes**. Stop reaction by adding 100 µL of 1N phosphoric acid to each well, including 1A-3A, in the same order of pipetting used for the TMB substrate solution.
    Note: Other strong acids typically used as stop solutions in ELISA may be substituted for 1N phosphoric acid. If bubbles are present in the wells, agitate slightly before reading.
12. Read the plate at 450 nm.

3. Calculation of Results

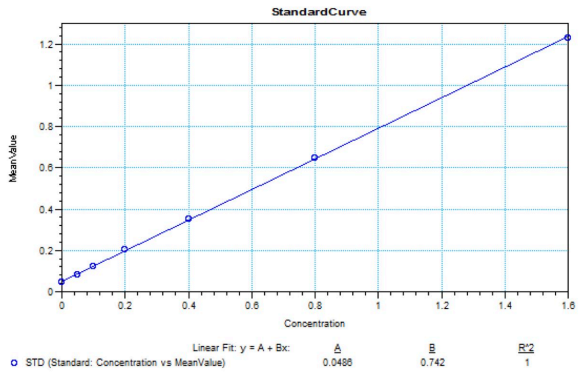
1. Calculate the mean absorbance value for the plate blank wells (A1-A3) and subtract from all remaining wells on the plate (including the 0 ng/mL standard curve.) Determine the average absorbance value for each Standard concentration and all Test Samples.

Note: Method of calculation for Standard Curve should be based on internal standards. Other curve fits may be used as deemed appropriate.

2. Calculate the standard curve:

<u>Linear Fit</u>
Plot each Standard Curve concentration (ng/mL Protein A) on the x-axis and the corresponding mean absorbance value on the y-axis. Using linear regression, calculate the best-fitting straight line through the points of the standard curve (See Figure 3.1)

Figure 3.1 Standard Curve Linear Regression



<u>4-Parameter Fit</u>
Logistic curve-fitting software can also be used to construct the standard curve. Such a fit is the acknowledged reference model for sigmoidal immunoassay data.[6,7] The regression line can be used to determine the rProtein A™ concentration [PA] for the samples.

[PA] x Sample Diluent = C (ng/mL)

To determine the ng/mg (ppm) of Protein A in each sample well, use the following formula:

$$\text{ng/mg} = \frac{\text{Mean concentration [ng/mL]}}{\text{mg/mL of Antibody per well (e.g. 0.125 mg/mL)}}$$

Specificity
This Protein A ELISA Kit (9000-1) is supplied with Repligen's recombinant Protein A Standard Solution. For the behavior of the kit with of other variants of Protein A, please contact Technical Support by calling +1 (781) 250-0111 (option 3).

4. Troubleshooting

Problem: Pipetting enough of required reagent

Possible Cause	Remedy
Splashing of reagent on sides or cap of reagent tube during mixing, shipping or handling.	Centrifuge tube briefly.

Problem: Inconsistent results between sample dilutions

Possible Cause	Remedy
Antibody was not fully equilibrated in PBS, pH 7.0 - 7.4, before assay.	Re-dialyze sample in PBS. Ensure pH is 7.0 - 7.4 and re-run assay.
The antibody concentration in the undiluted sample was > 0.5 mg per mL.	Ensure antibody concentration is < 0.5 mg/mL.

Problem: "Outliers," where one replicate has an abnormally high or low absorbance value

Possible Cause	Remedy
Small amount of peroxidase conjugate left on the plate before color development. (i.e. wells were not thoroughly washed)	Discard outliers and average duplicates. Ensure thorough washing in any subsequent ELISA testing.

Problem: Color development time to reach 1.0 AU is > 4-5 minutes

Possible Cause	Remedy
TMB solution, Reagent E, was not at room temperature before adding to wells.	Solution can be warmed before adding to wells.
Room temperature too low, or too cool.	Use incubator set at 68-77°F (20-25°C) for all incubations, or develop longer than 4 minutes.

Problem: Background signal is > 0.150

Possible Cause	Remedy
Color development for TMB substrate was > 4 minutes.	Start timer immediately after adding TMB substrate to 1.6 ng/mL Standard wells.
Temperature of TMB substrate > 77°F (25°C).	Store TMB in a location that is between 68-77°F (20-25°C) until use.
Insufficient plate washing.	Ensure plate was washed 4 times.

Problem: O.D. values consistently high for all samples, or Low recovery of Protein A in samples

Possible Cause	Remedy
Buffer component interference	Buffer-exchange sample into neutral buffer, or perform a greater fold dilution into neutral buffer (Section 2.2)

5. Additional References

(1) H. Fey and G. Burkhard, (1981) "Measurement of Staphylococcal Protein A and Detection of Protein A-Carrying Staphylococcus Strains by a Competitive ELISA method" J. Immunol. Methods 47: 99-107.

(2) A. Warnes, A. Walkland and J.R. Stephenson, (1986) "Development of an Enzyme-Linked Immunosorbent Assay for Staphylococcal Protein A Produced in Escherichia coli by pUC8-based Plasmids Containing the Staphylococcus aureus Cowan I protein A Gene" J. Immunol. Methods 93:63-70.

(3) M.T. Dertzbaugh, M.C. Flickinger and W.B. Lebherz III, (1985) "An Enzyme Immunoassay for the Detection of Staphylococcal Protein A in Affinity-Purified Products" J. Immunol. Methods 83: 169-177.

(4) J.W. Bloom, M.F. Wong and G. Mitra, (1989) "Detection and Reduction of Protein A Contamination in Immobilized Protein A-Purified Monoclonal Antibody Preparations" J. Immunol. Methods 117: 83-89.

(5) S.M. Knicker, A.T. Profy, (1991) "Immunoassay to Measure Staphylococcal Protein A in the Presence of Murine Immunoglobulins" J. Immunol. Methods 142: 53-59.

(6) Dudley, R.A., P. Edwards, et al. (1985) "Guidelines for immunoassay data processing." Clin Chem 31(8): 1264-71.

(7) Smith, W.C. and G.S. Sittampalam (1998) "Conceptual and statistical issues in the validation of analytic dilution assays for pharmaceutical applications." J Biopharm Stat 8(4): 509-32.

6. Safety Data Sheet

Reagent D – Component of rProtein A™ Kit 9000-1

This Safety Data Sheet was prepared according to Regulation (EC) No. 1907/2006 (REACH) as amended SDS ID: REP-002.

For additional languages please refer to: www.repligen.com/resources/quality-documents/

Section 1 – Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Material Name:	Reagent D
Contains:	0.003% CMIT/MIT
Product Description:	Kit Component
Chemical Family:	Isothiazolinones
Substance Registration Number(s):	This material is imported in amounts <1 ton/year. This product and the other components are not subject to REACH legislation.

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses:	Detection and quantification of Protein A
Uses advised against:	R&D use only

1.3 Details of the supplier of the safety data sheet

Repligen Corporation.
41 Seyon Street, Building 1 Suite 100
Waltham, MA 02453
Phone: 1 (800) 622-2259
E-mail: sales@repligen.com
Fax: 1 (781) 250-0115

1.4 Emergency telephone number: 1 (800) 622-2259

Section 2 – Hazards Identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 [CLP] Skin Sensitization - Category 1

2.2 Label elements

Labeling according to Regulation (EC) No. 1272/2008 [CLP]

Hazard Symbols:

Signal word: Warning

Hazard statements: GHS code H317 May cause allergic skin reaction.

Precautionary statements

Prevention:

GHS code P280	Wear protective gloves/protective clothing/eye protection/face protection.
GHS code P261	Avoid breathing dust/fume/gas/mist/vapours/spray.

Response:

GHS code P305 +P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
GHS code P302+P352	IF ON SKIN: Wash with plenty of soap and water.
GHS code P333+P313	If skin irritation or rash occurs: Get medical attention.
GHS code P362+P364	Take off contaminated clothing and wash before reuse.

Storage: None needed according to classification criteria**.**

Disposal:

GHS code P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

2.3 Other hazards None known.

Section 3 – Composition / Information on Ingredients

CAS EC No Registration No	Component Name Synonyms	1272/2008 (CLP)	Percent
55965-84-9 -- --	5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone	Acute Tox. (Oral) 3 - H301 Acute Tox. (Vapour) 3 - H331 Acute Tox. (Gas) 3 - H331 Acute Tox. (Dermal) 3 - H311 Acute Tox. (Dust/Mist) 3 - H331 Skin Corr. 1B - H314 Skin Sens. 1 - H317 Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410	0.003

Full text of H- and EUH-statements: see section 16.

Section 4 – First Aid Measures

4.1 Description of first aid measures

Inhalation:	If adverse effects occur, remove to uncontaminated area. Get immediate medical attention.
Skin:	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse. Immediately call a POISON CENTER or doctor.
Eyes:	Flush eyes with plenty of water for at least 15 minutes. If eye irritation persists: Get medical attention.
Ingestion:	If swallowed, get medical attention. Do NOT induce vomiting.

4.2 Most Important Symptoms/Effects

Acute:	May cause an allergic skin reaction.
Delayed:	No information on significant adverse effects.

4.3 Indication of Immediate Medical Attention and Special Treatment

Treat symptomatically and supportively.

Section 5 – Firefighting Measures

5.1 Extinguishing media

Suitable extinguishing media:	Use foam, dry chemical, CO_2, or water spray.
Unsuitable Extinguishing Media:	None known.

5.2 Special hazards arising from the substance or mixture

None known.

Combustion:	Decomposition products include oxides of carbon and low molecular weight hydrocarbons.

5.3 Advice for firefighters	Fire fighters should wear full-face, self-contained breathing apparatus and impervious protective clothing. Firefighters should avoid inhaling any combustion products.
Firefighting Measures:	Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

Section 6 – Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures:

Wear personal protective clothing and equipment, see Section 8.

6.2 Environmental precautions:	Avoid release to the environment. Do not allow to enter into ground-water, surface water or drains.

6.3 Methods and Materials for Containment and Cleaning Up:

Contain the discharged material with an inert absorbent material. Isolate hazard area. Keep unnecessary personnel away.

6.4 Reference to other sections:	Safe handling: see section 7. Personal protection equipment (PPE): see section 8. Disposal: see section 13.

Section 7 – Handling and Storage

7.1 Precautions for safe handling:	Use only outdoors or in a well-ventilated area. Do not eat, drink or smoke when using this product. Wear eye/face protection. Wash thoroughly after handling.

7.2 Conditions for safe storage, including any incompatibilities:

Keep container tightly closed. Keep away from heat/sparks/open flame/hot surfaces - No smoking. Store at 2-8 °C.

Incompatible Materials:	Strong oxidizing agents, peroxides, acid, alkali
7.3 Specific end use(s):	Research and Development (R&D) Use Only.

Section 8 – Exposure Controls/Personal Protection

8.1 Control parameters
Component Exposure Limits

5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone	55965-84-9
Austria:	0.05 mg/m3 TWA [TMW]
	skin notation
	Skin sensitizer

Component Biological Exposure Limits: None of this product's components are on the list.
Derived No Effect Levels (DNELs): No DNELs available.
Predicted No Effect Concentrations (PNECs): No PNECs available.

8.2 Exposure Controls
Engineering controls: Provide adequate ventilation. Ensure compliance with applicable exposure limits.
Eye/face protection: Wear safety googles with a face shield (EN 166).
Skin Protection: Wear suitable protective clothing. Wash contaminated clothing before reuse (EN ISO 6529).
Respiratory Protection: If engineering controls do not maintain airborne concentrations to a negligible level, an approved respirator must be worn (EN 137).
Glove Recommendations: Wear suitable gloves (EN 374).

Section 9 – Physical and Chemical Properties

9.1 Information on basic physical and chemical properties

Appearance	Colorless liquid	Physical State	Not available
Odor	Not available	Color	Colorless, clear to light yellow
Odor Threshold	Not available	pH	7
Melting Point	Not available	Boiling Point	Not available
Boiling Point Range	Not available	Freezing point	Not available
Evaporation Rate	Not available	Flammability (solid, gas)	Not available
Autoignition Temperature	Not available	Flash Point	Not available
Lower Explosive Limit	Not available	Decomposition temperature	Not available
Upper Explosive Limit	Not available	Vapor Pressure	Not available
Vapor Density (air=1)	Not available	Specific Gravity (water=1)	Not available

Water Solubility	(soluble)	Partition coefficient: n-octanol/water	Not available
Viscosity	Not available	Solubility (Other)	Not available
Density	Not available	Physical Form	liquid
Molecular Weight	Not available		

9.2 Other information — No additional information is available.

Section 10 – Stability and Reactivity

10.1 Reactivity: — No reactivity hazard is expected.

10.2 Chemical stability: — Stable at normal temperatures and pressure.

10.3 Possibility of hazardous reactions: — Will not polymerize.

10.4 Conditions to avoid: — Avoid contact with incompatible materials.

10.5 Incompatible materials: — Strong oxidizing agents, peroxides, acids, alkalis

10.6 Hazardous decomposition Products — Decomposition products include oxides of carbon and low molecular weight hydrocarbons.

Section 11 – Toxicological Information

11.1 Information on toxicological effects

Component Analysis - LD50/LC50 — The components of this material have been reviewed in various sources and the following selected endpoints are published:

5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone (55965-84-9)
Oral LD50 Rat 53 mg/kg

Product Toxicity Data
Acute Toxicity Estimate

Dermal	> 2000 mg/kg
Oral	> 2000 mg/kg

Irritation/Corrosivity Data: — May cause an allergic skin reaction.
Respiratory Sensitization: — No data available.
Dermal Sensitization: — May cause an allergic skin reaction.
Germ Cell Mutagenicity: — No data available.
Component Carcinogenicity: — None of this product's components are listed by IARC or DFG.
Reproductive toxicity: — No data available.
Specific Target Organ Toxicity – Single Exposure: — No target organs identified.
Specific Target Organ Toxicity – Repeated Exposure: — No target organs identified.
Aspiration hazard — No data available.

Section 12 – Ecological Information

12.1 Toxicity:
Component Analysis – Aquatic Toxicity:
No LOLI ecotoxicity data are available for this product's components.

12.2 Persistence and degradability: No information available for the product.

12.3 Bioaccumulative potential: No information available for the product.

12.4 Mobility in soil: No information available for the product.

12.5 Results of PBT and vPvB assessment:
No information available for the product.

Section 13 – Disposal Considerations

13.1 Waste treatment methods: Dispose of contents/container in accordance with local/regional/national/international regulations.
Recycle if possible. EWC-code: 18 02 05*.
No data available.
Release to the environment or sewage system is prohibited.
Dispose in accordance with all applicable regulations.

Section 14 – Transport Information

		ADR	RID	ICAO	IATA	ADN	IMDG
14.1	UN Number	Not regulated	Not regulated	Not regulated	Not regulated	Not regulated	Not regulated
14.2	UN Proper Shipping Name	--	--	--	--	--	--
14.3	Transport Hazard Class(es)	--	--	--	--	--	--
14.4	Packing Group	--	--	--	--	--	--
14.5	Environmental Hazards	--	--	--	--	--	--
14.6	Special Precautions For User	--	--	--	--	--	--
14.7	Transport in Bulk According to Annex II of MARPOL and the IBC Code	--	--	--	--	--	--
14.8	Additional information	--	--	--	--	--	--

Component Marine Pollutants (IMDG): Not a marine pollutant.
International Bulk Chemical Code: This material does not contain any chemicals required by the IBC Code to be identified as dangerous chemicals in bulk.

Section 15 – Regulatory Information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

EU - REACH (1907/2006) - Annex XIV List of Substances Subject to Authorization
No components of this material are listed.
EU - REACH (1907/2006) - Article 59(1) Candidate List of Substances Subject to Authorization
No components of this material are listed.
EU - REACH (1907/2006) - Annex XVII Restrictions of Certain Dangerous Substances, Mixtures and Articles. No components of this material are listed.
EU - Substances Depleting the Ozone layer (1005/2009). No components of this material are listed.
EU - Persistent Organic Pollutants (850/2004). No components of this material are listed.
EU - Export and Import Restrictions (689/2008) - Chemicals and Articles Subject to Export Ban. No components of this material are listed.
EU - Seveso III Directive (2012/18/EU) - Qualifying Quantities of Dangerous Substances. No components of this material are listed.
EU - Plant Protection Products (1107/2009/EC). No components of this material are listed
EU - Biocides (528/2012/EU). No components of this material are listed.
EU – Water Framework Directive (2000/60/EC). No components of this material are listed.
EU - Limitation of Emissions of Volatile Organic Compounds Due to the Use of Organic Solvents in Certain Activities and Installations (1999/13/EC). No components of this material are listed.
Germany Regulations
Germany Water Classification - Product non-hazardous to water (nwg). Germany Water Classification - Component 5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone (55965-84-9) ID Number 2959, hazard class 3 - severe hazard to waters
Denmark Regulations - No components of this material are listed.

Component Analysis - Inventory
5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone (55965-84-9)

US	CA	EU	AU	PH	JP - ENCS	JP - ISHL	KR - KECI/KECL	KR - TCCA	CN	NZ	MX	TW
No	DSL	No	No	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes

15.2 Chemical Safety Assessment
No chemical safety assessment has been carried out for the substance/mixture.

Section 16 – Other Information

16.1 Indication of changes New SDS: 12 June 2017

16.2 Key / Legend:
ACGIH - American Conference of Governmental Industrial Hygienists; ADR - European Road Transport; AU - Australia; BOD - Biochemical Oxygen Demand; C - Celsius; CA - Canada; CA/MA/MN/NJ/PA - California/Massachusetts/Minnesota/New Jersey/Pennsylvania*; CAS - Chemical Abstracts Service; CFR - Code of Federal Regulations (US); CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act; CLP - Classification, Labelling, and Packaging; CN - China; CPR - Controlled Products Regulations; DFG - Deutsche Forschungsgemeinschaft; DOT - Department of Transportation; DSD - Dangerous Substance Directive; DSL - Domestic Substances List; EC – European Commission; EEC - European Economic Community; EIN - European Inventory of (Existing Commercial Chemical Substances); EINECS - European Inventory of Existing Commercial Chemical Substances; ENCS - Japan Existing and New Chemical Substance Inventory; EPA - Environmental Protection Agency; EU - European Union; F - Fahrenheit; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; ICAO - International Civil Aviation Organization; IDL - Ingredient Disclosure List; IDLH - Immediately Dangerous to Life and Health; IMDG - International Maritime Dangerous Goods; ISHL - Japan Industrial Safety and Health Law; IUCLID - International Uniform Chemical Information Database; JP - Japan; Kow - Octanol/water partition coefficient; KECI - Korea Existing Chemicals Inventory; KECL – Korea Existing Chemicals List; KR - Korea; LD50/LC50 - Lethal Dose/ Lethal Concentration; LEL - Lower Explosive Limit; LLV - Level Limit Value; LOLI - List Of LIsts™ - ChemADVISOR's Regulatory Database; MAK - Maximum Concentration Value in the Workplace; MEL - Maximum Exposure Limits; MX – Mexico; NDSL – Non-Domestic Substance List (Canada); NFPA - National Fire Protection Agency; NIOSH - National Institute for Occupational Safety and Health; NJTSR - New Jersey Trade Secret Registry; NTP - National Toxicology Program; NZ - New Zealand; OSHA - Occupational Safety and Health Administration; PEL- Permissible Exposure Limit; PH - Philippines; RCRA - Resource Conservation and Recovery Act; REACH- Registration, Evaluation, Authorisation, and restriction of Chemicals; RID - European Rail Transport; SARA - Superfund Amendments and Reauthorization Act; STEL - Short-term Exposure Limit; TCCA – Korea Toxic Chemicals Control Act; TDG - Transportation of Dangerous Goods; TLV - Threshold Limit Value; TSCA - Toxic Substances Control Act; TW – Taiwan; TWA - Time Weighted Average; UEL - Upper Explosive Limit; UN/NA - United Nations /North American; US - United States; VLE - Exposure Limit Value (Mexico); WHMIS - Workplace Hazardous Materials Information System (Canada)

16.3 Key literature references and sources for data
Available upon request.

16.4 Methods Used for Classification of Mixture According to Regulation (EC) No 1272/2008
Available upon request.

16.5 Relevant H- and EUH-phrases
(Number and full text) and Notes:
H301 Toxic if swallowed
H311 Toxic in contact with skin
H314 Causes severe skin burns and eye damage
H317 May cause allergic skin reaction
H331 Toxic if inhaled
H400 Very toxic to aquatic life
H410 Very toxic to aquatic life with long lasting effects

16.6 Training advice: Read the Safety Data Sheet before handling product.

16.7 Further Information

Disclaimer:
Supplier gives no warranty whatsoever, including the warranties of merchantability or of fitness for a particular purpose. Any product purchased is sold on the assumption the purchaser shall determine the quality and suitability of the product. Supplier expressly disclaims any and all liability for incidental, consequential or any other damages arising out of the use or misuse of this product. No information provided shall be deemed to be a recommendation to use any product in conflict with any existing patent rights.

7. Safety Data Sheet

Reagent A (CPR1101) – Component of rProtein A™ Kit 9000-1

This Safety Data Sheet was prepared according to Regulation (EC) No. 1907/2006 (REACH) as amended SDS ID: REP-002.

For additional languages please refer to: www.repligen.com/resources/quality-documents/

Section 1 – Identification of the substance/mixture and of the company/undertaking

1.1 Product identifier

Material Name:	Reagent A (CPR1101)
Contains:	Sodium Acetate
Product Description:	Kit Component
Substance Registration Number(s):	This material is imported in amounts <1 ton/year. This product and the other components are not subject to REACH legislation.

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses:	Detection and quantification of Protein A
Uses advised against:	R&D use only

1.3 Details of the supplier of the safety data sheet

Repligen Corporation
41 Seyon Street, Building 1 Suite 100
Waltham, MA 02453
Phone: 1 (800) 622-2259
E-mail: sales@repligen.com
Fax: 1 (781) 250-0115

1.4 Emergency telephone number: 1 (800) 622-2259

Section 2 – Hazards Identification

2.1 Classification of the substance or mixture
Classification according to Regulation (EC) No 1272/2008 [CLP]
Skin Corrosion/Irritation - Category 2

2.2 Label elements
Labeling according to Regulation (EC) No. 1272/2008 [CLP]

Hazard Symbols:

Signal word: Warning

Hazard statements: GHS code H315: Causes skin irritation.

Precautionary statements

Prevention:

GHS code P280 Wear protective gloves.

GHS code P264 Wash thoroughly after handling.

Response:

GHS code P305 +P351 +P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

GHS code P302+P352 IF ON SKIN: Wash with plenty of soap and water.

GHS code P362+P364: Take off contaminated clothing and wash before reuse.

Storage: None needed according to classification criteria.

Disposal: GHS code P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

2.3 Other hazards None known.

Section 3 – Composition / Information on Ingredients

CAS EC No Registration No	Component Name Synonyms	1272/2008 (CLP)	Percent
127-09-3 204-823-8 --	Sodium acetate	Skin Irrit. 2 - H315	15
9005-64-5 500-018-3	Polyoxyethylene sorbitan monolaurate	--	0.1

* Self-classification. Full text of H- and EUH-statements: see section 16.

Section 4 – First Aid Measures

4.1 Description of first aid measures

Inhalation: If adverse effects occur, remove to uncontaminated area. Get immediate medical attention.

Skin: IF ON SKIN: Wash with plenty of soap and water. If skin irritation occurs: Get medical attention. Take off contaminated clothing and wash before reuse.

Eyes: Flush eyes with plenty of water for at least 15 minutes. If eye irritation persists: Get medical attention.

Ingestion: If swallowed, get medical attention. Do NOT induce vomiting.

4.2 Most Important Symptoms/Effects

Acute: Causes skin irritation.

Delayed: No information on significant adverse effects.

4.3 Indication of Immediate Medical Attention and Special Treatment

Treat symptomatically and supportively.

Section 5 – Firefighting Measures

5.1 Extinguishing media

Suitable extinguishing media: Use foam, dry chemical, CO_2, or water spray.

Unsuitable Extinguishing Media: None known.

5.2 Special hazards arising from the substance or mixture

May explode if heated in closed container.

Combustion: Decomposition products include oxides of carbon and low molecular weight hydrocarbons.

5.3 Advice for firefighters: Fire-fighters should wear full-face, self-contained breathing apparatus and impervious protective clothing. Firefighters should avoid inhaling any combustion products.

Fire Fighting Measures: Move container from fire area if it can be done without risk. Avoid inhalation of material or combustion by-products. Stay upwind and keep out of low areas.

Section 6 – Accidental Release Measures

6.1 Personal precautions, protective equipment and emergency procedures:

Wear personal protective clothing and equipment, see Section 8.

6.2 Environmental precautions: Avoid release to the environment. Do not allow to enter into ground-water, surface water or drains.

6.3 Methods and Materials for Containment and Cleaning Up:

Contain the discharged material with an inert absorbent material. Isolate hazard area. Keep unnecessary personnel away.

6.4 Reference to other sections: Safe handling: see section 7. Personal protection equipment (PPE): see section 8. Disposal: see section 13.

Section 7 – Handling and Storage

7.1 Precautions for safe handling: Use only outdoors or in a well-ventilated area. Do not eat, drink or smoke when using this product. Wear eye protection. Wash thoroughly after handling.

7.2 Conditions for safe storage, including any incompatibilities:

Keep container tightly closed. Keep away from heat/sparks/open flame/hot surfaces - No smoking. Store at 2-8 °C.

Incompatible Materials: Strong oxidizing agents, peroxides, acid, alkali

7.3 Specific end use(s): Detection and quantification of Protein A. R&D Use Only.

Section 8 – Exposure Controls/Personal Protection

8.1 Control parameters

Component Exposure Limits: EU, Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Netherlands, Portugal, Spain, Sweden and United Kingdom have not developed exposure limits for any of this product's components.

Component Biological Exposure Limits: None of this product's components are on the list.

Derived No Effect Levels (DNELs): No DNELs available.

Predicted No Effect Concentrations (PNECs): No PNECs available.

8.2 Exposure Controls

Engineering controls: Provide adequate ventilation. Ensure compliance with applicable exposure limits.

Eye/face protection: Wear safety googles with a faceshield (EN 166).

Skin Protection: Wear suitable protective clothing. Wash contaminated clothing before reuse (EN ISO 6529).

Respiratory Protection: If engineering controls do not maintain airborne concentrations to a negligible level, an approved respirator must be worn (EN 137).

Glove Recommendations: Wear suitable gloves (EN 374).

Environmental exposure controls: Avoid release to the environment.

Section 9 – Physical and Chemical Properties

9.1 Information on basic physical and chemical properties

Appearance	Colorless liquid	**Physical State**	Not available
Odor	strong ,vinegar-like	**Color**	Colorless liquid
Odor Threshold	Not available	**pH**	3
Melting Point	Not available	**Boiling Point**	Not available
Boiling Point Range	Not available	**Freezing point**	Not available
Evaporation Rate	Not available	**Flammability (solid, gas)**	Not available
Autoignition Temperature	Not available	**Flash Point**	Not available
Lower Explosive Limit	Not available	**Decomposition temperature**	Not available
Upper Explosive Limit	Not available	**Vapor Pressure**	Not available
Vapor Density (air=1)	Not available	**Specific Gravity (water=1)**	Not available
Water Solubility	(soluble)	**Partition coefficient: n-octanol/water**	Not available
Viscosity	Not available	**Solubility (Other)**	Not available
Density	Not available	**Physical Form**	liquid
Molecular Weight	Not available		

9.2 Other information: No additional information is available.

Section 10 – Stability and Reactivity

10.1 Reactivity: May be corrosive to metals.

10.2 Chemical stability: Stable at normal temperatures and pressure.

10.3 Possibility of hazardous reactions: Will not polymerize.

10.4 Conditions to avoid: Avoid contact with incompatible materials. Avoid heat, flames, sparks and other sources of ignition

10.5 Incompatible materials: Strong oxidizing agents, peroxides, acids, alkalis

10.6 Hazardous decomposition Products: Decomposition products include oxides of carbon and low molecular weight hydrocarbons.

Section 11 – Toxicological Information

11.1 Information on toxicological effects

Component Analysis - LD50/LC50 The components of this material have been reviewed in various sources and the following selected endpoints are published:

Sodium acetate (127-09-3)

Oral LD50 Rat 3530 mg/kg
Dermal LD50 Rabbit >10 g/kg
Inhalation LC50 Rat >30 g/m3 1 h

Polyoxyethylene sorbitan monolaurate (9005-64-5)

Oral LD50 Rat 36700 µL/kg

Product Toxicity Data
Acute Toxicity Estimate

Dermal	> 2000 mg/kg
Oral	> 2000 mg/kg

Irritation/Corrosivity Data: Causes skin irritation.
Respiratory Sensitization: No data available.
Dermal Sensitization: No data available.
Germ Cell Mutagenicity: No data available.
Component Carcinogenicity: None of this product's components are listed by IARC or DFG.
Reproductive toxicity: No data available.
Specific Target Organ Toxicity – Single Exposure: No target organs identified.
Specific Target Organ Toxicity – Repeated Exposure: No target organs identified.
Aspiration hazard No data available.

Section 12 – Ecological Information

12.1 Toxicity:
Component Analysis – Aquatic Toxicity:

Sodium acetate	**127-09-3**
Invertebrate:	EC50 48 h Daphnia magna >1000 mg/L IUCLID

12.2 Persistence and degradability: No information available for the product.

12.3 Bioaccumulative potential: No information available for the product.

12.4 Mobility in soil: No information available for the product.

12.5 Results of PBT and

vPvB assessment: No information available for the product.

Section 13 – Disposal Considerations

13.1 Waste treatment methods: Waste disposal according to directive 2008/98/EC, covering waste and dangerous waste.
Waste codes/waste designations according to LoW. EWC-code: 18 02 05*.
No data specific data available.
Release to the environment or sewage system is prohibited.
Recycle if possible. Dispose of material in accordance with all local, regional, national and international regulations.

Section 14 – Transport Information

		ADR	RID	ICAO	IATA	ADN	IMDG
14.1	UN Number	Not regulated	Not regulated	Not regulated	Not regulated	Not regulated	Not regulated
14.2	UN Proper Shipping Name	--	--	--	--	--	--
14.3	Transport Hazard Class(es)	--	--	--	--	--	--
14.4	Packing Group	--	--	--	--	--	--
14.5	Environmental Hazards	--	--	--	--	--	--
14.6	Special Precautions For User	--	--	--	--	--	--
14.7	Transport in Bulk According to Annex II of MARPOL and the IBC Code	--	--	--	--	--	--
14.8	Additional information	--	--	--	--	--	--

Component Marine Pollutants (IMDG): Not a marine pollutant.
International Bulk Chemical Code: This material does not contain any chemicals required by the IBC Code to be identified as dangerous chemicals in bulk.

Section 15 – Regulatory Information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

EU - REACH (1907/2006) - Annex XIV List of Substances Subject to Authorization. - No components of this material are listed.
EU - REACH (1907/2006) - Article 59(1) Candidate List of Substances Subject to Authorization. - No components of this material are listed.
EU - REACH (1907/2006) - Annex XVII Restrictions of Certain Dangerous Substances, Mixtures and Articles. - No components of this material are listed.
EU - Substances Depleting the Ozone layer (1005/2009). - No components of this material are listed
EU - Persistent Organic Pollutants (850/2004). - No components of this material are listed
EU - Export and Import Restrictions (689/2008) - Chemicals and Articles Subject to Export Ban. - No components of this material are listed
EU - Seveso III Directive (2012/18/EU) - Qualifying Quantities of Dangerous Substances. - No components of this material are listed
EU - Plant Protection Products (1107/2009/EC). - No components of this material are listed
EU - Biocides (528/2012/EU)

Sodium acetate	**127-09-3**
Active Substances	Category 1 (E 262)

EU – Water Framework Directive (2000/60/EC). - No components of this material are listed
EU - Limitation of Emissions of Volatile Organic Compounds Due to the Use of Organic Solvents in Certain Activities and Installations (1999/13/EC). - No components of this material are listed
EU Detergent Regulation 648/2004/EC. - No components of this material are listed
Germany Regulations
Germany Water Classification - Product hazard class 1 - low hazard to waters * Self-classification
Germany Water Classification - Component Sodium acetate (127-09-3) ID Number 367 , hazard class 1 - low hazard to waters
Polyoxyethylene sorbitan monolaurate (9005-64-5) ID Number 3692, hazard class 1 - low hazard to waters
Denmark Regulations - No components of this material are listed.

Component Analysis - Inventory

Sodium acetate (127-09-3)

US	CA	EU	AU	PH	JP - ENCS	JP - ISHL	KR KECI - Annex 1	KR KECI - Annex 2	KR - REACH CCA	CN	NZ	MX	TW
Yes	DSL	EIN	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes

Polyoxyethylene sorbitan monolaurate (9005-64-5)

US	CA	EU	AU	PH	JP - ENCS	JP - ISHL	KR KECI - Annex 1	KR KECI - Annex 2	KR - REACH CCA	CN	NZ	MX	TW
Yes	DSL	No	Yes	Yes	No	No	Yes	No	No	Yes	Yes	Yes	Yes

15.2 Chemical Safety Assessment

No chemical safety assessment has been carried out for the substance/mixture.

Section 16 – Other Information

16.1 Indication of changes New SDS: 12 June 2017

16.2 Key / Legend:
ACGIH - American Conference of Governmental Industrial Hygienists; ADR - European Road Transport; AU - Australia; BOD - Biochemical Oxygen Demand; C - Celsius; CA - Canada; CA/MA/MN/NJ/PA - California/Massachusetts/Minnesota/New Jersey/Pennsylvania*; CAS - Chemical Abstracts Service; CFR - Code of Federal Regulations (US); CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act; CLP - Classification, Labelling, and Packaging; CN - China; CPR - Controlled Products Regulations; DFG - Deutsche Forschungsgemeinschaft; DOT - Department of Transportation; DSD - Dangerous Substance Directive; DSL - Domestic Substances List; EC – European Commission; EEC - European Economic Community; EIN - European Inventory of (Existing Commercial Chemical Substances); EINECS - European Inventory of Existing Commercial Chemical

Substances; ENCS - Japan Existing and New Chemical Substance Inventory; EPA - Environmental Protection Agency; EU - European Union; F - Fahrenheit; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; ICAO - International Civil Aviation Organization; IDL - Ingredient Disclosure List; IDLH - Immediately Dangerous to Life and Health; IMDG - International Maritime Dangerous Goods; ISHL - Japan Industrial

Safety and Health Law; IUCLID - International Uniform Chemical Information Database; JP - Japan; Kow - Octanol/water partition coefficient; KECI - Korea Existing Chemicals Inventory; KECL – Korea Existing Chemicals List; KR - Korea; LD50/LC50 - Lethal Dose/ Lethal Concentration; LEL - Lower Explosive Limit; LLV - Level Limit Value; LOLI - List Of LIsts™ - ChemADVISOR's Regulatory Database; MAK - Maximum Concentration Value in the Workplace; MEL - Maximum Exposure Limits; MX – Mexico; NDSL – Non-Domestic Substance List (Canada); NFPA - National Fire Protection Agency; NIOSH - National Institute for Occupational Safety and Health; NJTSR - New Jersey Trade Secret Registry; NTP - National Toxicology Program; NZ - New Zealand; OSHA - Occupational Safety and Health Administration; PEL- Permissible Exposure Limit; PH - Philippines; RCRA - Resource Conservation and Recovery Act; REACH- Registration, Evaluation, Authorisation, and restriction of Chemicals; RID - European Rail Transport; SARA - Superfund Amendments and Reauthorization Act; STEL - Short-term Exposure Limit; TCCA – Korea Toxic Chemicals Control Act; TDG - Transportation of Dangerous Goods; TLV - Threshold Limit Value; TSCA - Toxic Substances Control Act; TW – Taiwan; TWA - Time Weighted Average; UEL - Upper Explosive Limit; UN/NA - United Nations /North American; US - United States; VLE - Exposure Limit Value (Mexico); WHMIS - Workplace Hazardous Materials Information System (Canada)

16.3 Key literature references and sources for data:
Available upon request.

16.4 Methods Used for Classification of Mixture
According to Regulation (EC) No 1272/2008 Available upon request.

16.5 Relevant H- and EUH-phrases H315 Causes skin irritation.

16.6 Training advice: Read the Safety Data Sheet before handling product.

16.7 Further Information

Disclaimer:

Supplier gives no warranty whatsoever, including the warranties of merchantability or of fitness for a particular purpose. Any product purchased is sold on the assumption the purchaser shall determine the quality and suitability of the product. Supplier expressly disclaims any and all liability for incidental, consequential or any other damages arising out of the use or misuse of this product. No information provided shall be deemed to be a recommendation to use any product in conflict with any existing patent rights.