QUICK REFERENCE GUIDE

Reagent Preparation (Before Assay)
1. Make 100 mM acetic acid: 100 mL purified water + 0.575 mL glacial acetic acid.
2. Make 1X diluent (Reagent A): 16 mL purified water + 4 mL Reagent A.
3. Prepare PBS Buffer solution: 1 L purified water + 1x PBS packet.
4. Make PBS-Tween 20: 1 L purified water + 1x PBS packet + 0.5 mL Tween 20.

Setup
1. Design experiment and set up microtiter wells as needed. For more information see Section 2.2 of this user guide.
2. Remove the ELISA plate from 2 - 8°C storage and allow it to equilibrate to room temperature for at least 10 minutes.
3. Remove TMB (Reagent E) from 2-8°C storage and allow to equilibrate to room temperature.

Sample Preparation
1. Samples containing LONG®R³ IGF-I within the standard curve range may be assayed without dilution.
2. Samples containing greater than 40 ng/mL LONG®R³ IGF-I must be diluted using 1x Reagent A before analysis.

Reagent Preparation (During Assay)
3. Use TMB (Reagent E) as is: No dilution required.
ELISA Procedure

1. Wash the plate twice with 1X PBS before use.
2. Add 100 µL/well Standards and Samples to the plate*.
3. Incubate for 2 hours at room temperature.
4. Wash three times with PBS Tween 20.
5. Add 100 µL/well Detection Antibody to the plate*.
6. Incubate for 1 hour at room temperature.
7. Wash three times with PBS Tween 20.
8. Add 100 µL/well HRP- Streptavidin to the plate*.
9. Incubate for 30 minutes at room temperature.
10. Wash two times with PBS Tween 20 and once with 1X PBS.
11. Add 100 µL/well TMB to the plate*.
12. Develop for **15 minutes**, then stop with 100 µL/well 4 N sulfuric acid.
13. Read plate at 450 nm.

*Note: To ensure even dispersion, gently rotate the plate by hand about 10 times.

First-time kit users are encouraged to work through the complete User Guide.

---

**Standard Curve**

<table>
<thead>
<tr>
<th>Standard Curve</th>
<th>Reagent B</th>
<th>Tube B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Add 1 mL of 100 mM acetic acid to Reagent B</td>
<td>995 µL of 100 mM acetic acid + 5 µL of Reagent B</td>
</tr>
<tr>
<td>40 ng/mL</td>
<td>992 µL of 1X Diluent + 8 µL of Tube B</td>
<td></td>
</tr>
<tr>
<td>20 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 40 ng/mL</td>
<td></td>
</tr>
<tr>
<td>10 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 20 ng/mL</td>
<td></td>
</tr>
<tr>
<td>5 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 10 ng/mL</td>
<td></td>
</tr>
<tr>
<td>2.5 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 5 ng/mL</td>
<td></td>
</tr>
<tr>
<td>1.25 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 2.5 ng/mL</td>
<td></td>
</tr>
<tr>
<td>0.63 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 1.25 ng/mL</td>
<td></td>
</tr>
<tr>
<td>0.31 ng/mL</td>
<td>500 µL of 1X Diluent + 500 µL of 0.63 ng/mL</td>
<td></td>
</tr>
<tr>
<td>0 ng/mL</td>
<td>1,000 µL of 1X Diluent</td>
<td></td>
</tr>
</tbody>
</table>
LONG®R³ IGF-I ELISA Kit ELISA Kit (9547-1)

User Guide and Safety Data Sheet

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

USER GUIDE

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dilution........................................................................................................................................ 6
       Samples that contain greater than 40 ng/mL LONG®R³ IGF-I should be diluted in 1X Reagent A
before analysis. Dilute samples in LoBind tubes using a minimum number of dilution steps............. 6
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1. Overview of ELISA

LONG*R³ IGF-I is an analog of IGF-I (Insulin-like Growth Factor 1) which has been engineered for improved bioavailability compared to IGF-I. It binds to and activates the Type I IGF-I receptor, leading to improved cell growth and productivity. Quantitation of LONG*R³ IGF-I is important when developing cell culture processes to optimize the concentration of LONG*R³ IGF-I used and feeding strategies. Quantitation is also important when developing a purification process in order to demonstrate clearance.

Repligen LONG*R³ IGF-I Kit 9547-1 has been developed for the quantitation of LONG*R³ IGF-I in media and drug substance samples. As shown in Figure 1.1, the plate is coated with a monoclonal antibody to LONG*R³ IGF-I and then incubated with samples. A second biotinylated detection antibody is added, followed by Streptavidin-HRP (Horseradish Peroxidase). The plate is incubated with TMB substrate (3,3’,5,5’-Tetramethylbenzidine) for color development and stopped with sulfuric acid. Signal is proportional to the amount of LONG*R³ IGF-I in samples and standards.

Figure 1.1 - Schematic of LONG*R³ IGF-I Sandwich ELISA

1.1 Important points

1. Repligen Kit 9547-1 is designed for quantitation of LONG*R³ IGF-I. It has ~40% reactivity to IGF-I and no reactivity to insulin.
2. LONG*R³ IGF-I adsorbs to plastic and glass surfaces. Samples and standards should be prepared in low-binding tubes.
3. When preparing samples, avoid multiple dilutions and minimize the number of transfers to prevent adsorption.
4. When withdrawing LONG*R³ IGF-I Detection Antibody or Streptavidin-HRP from stock solutions draw up the correct volume in the pipette tip, expel it entirely, and then draw up the correct volume again. This corrects for any adsorptive losses on tips.

1.2 Reagents

Table 1.1  Reagents provided

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Description</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>Sample Diluent (5X) concentrate</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Reagent B</td>
<td>Reference Standard, Lyophilized solid</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Reagent C</td>
<td>Detection Antibody, Lyophilized solid</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Reagent D</td>
<td>Streptavidin-HRP (Horseradish Peroxidase) conjugate</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Reagent E</td>
<td>TMB Peroxidase substrate, contains 3, 3', 5, 5'-tetramethylbenzidine in buffer</td>
<td>2-8°C</td>
</tr>
<tr>
<td>LoBind Tubes</td>
<td>Used for Standard preparation</td>
<td>Ambient</td>
</tr>
<tr>
<td>ELISA Plate</td>
<td>96-well microtiter plate coated with anti-LR3 antibody, packed with desiccants</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
Table 1.2  Reagents, supplies, and equipment not provided with the kit

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>dH₂O or HPLC-Grade Water (preferred)</td>
<td>LoBind tubes (sample preparation)</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>4N sulfuric acid</td>
</tr>
<tr>
<td>Plate sealers</td>
<td>Micro-pipettors and 12-channel pipettor</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Pipette tips</td>
</tr>
<tr>
<td>15 and 50 mL plastic centrifuge tubes</td>
<td>Timer</td>
</tr>
<tr>
<td>Reagent reservoirs</td>
<td>Vortex mixer</td>
</tr>
<tr>
<td>10 and 25 mL Serological pipettes</td>
<td>ELISA plate reader with wavelength capability at 450 nm</td>
</tr>
</tbody>
</table>

2. Guide to Standard Preparation and Assay

2.1 Pre-Assay Reagent Preparation

100 mM acetic acid
Add 100 mL of purified water to an appropriately sized bottle, add 0.575 mL of glacial acetic acid. Mix by shaking to generate 100 mM acetic acid solution. Solution may be scaled down as needed.

1X Diluent
Dilute 4.0 mL of Reagent A (5X Sample Diluent) in 16 mL of purified water in a 50 mL plastic centrifuge tube. Invert tube 10 times for thorough mixing. To prevent foaming, do not use vortex mixer.

PBS Buffer Solution
Mix one PBS packet with 1 L of purified water. Mix by swirling or inversion.

PBS-Tween 20 Wash Solution
Mix one PBS packet with 1 L of purified water. Add 0.5 mL Tween 20. Mix by swirling or inversion.

2.2 Sample Preparation

Samples that contain LONG®R³ IGF-I within the standard curve range may be assayed without dilution.

Samples that contain greater than 40 ng/mL LONG®R³ IGF-I should be diluted in 1X Reagent A before analysis. Dilute samples in LoBind tubes using a minimum number of dilution steps.

2.3 Standard Preparation

LONG®R³ IGF-I Reference Standard

Note: LoBind tubes must be used for all dilutions of the LONG®R³ IGF-I Reference Standard.

Label one Eppendorf® LoBind 1.5 mL tube as Tube B. The final concentration of Tube B will be 5 µg/mL. Label eight Eppendorf® LoBind 1.5mL tubes with the concentrations of standard used in the ELISA: 40, 20, 10, 5, 2.5, 1.25, 0.63, and 0.31 ng/mL.

1. Reconstitute LONG®R³ IGF-I Reference Standard (Reagent B), by adding 1 mL of 100 mM acetic acid to the lyophilized Reference Standard vial for a final concentration of 1 mg/mL. Mix the tube by inverting 10 times. See FAQ section for information on storage and reuse.

2. Let it sit for 15 minutes before use.

3. Prepare the 40 ng/mL standard by adding 8 µL of Tube B to 992 µL of 1X Diluent for a final concentration of 40 ng/mL. Mix tube by inverting 10 times.
4. Use the 40 ng/mL tube as starting point for 2-fold serial dilutions. Add 500 µL of 1X Diluent to each of the tubes labeled 20, 10, 5, 2.5, 1.25, 0.63 and 0.31 ng/mL. Transfer 500 µL from the 40 ng/mL tube to the 20 ng/mL tube. Mix tube by inverting 10 times. Transfer 500 µL from the 20 ng/mL tube to the 10 ng/mL tube. Repeat this transfer method and inversion step for each tube, ending with the 0.31 ng/mL tube. Use 1X diluent for the blank wells. Store tubes at room temperature and use on same day as preparation.

![Preparations of standards](Figure 2.1)

2.4 Plate Set-up
Define the microtiter plate setup before starting the assay. It is recommended to run standards and samples in triplicate. A representative plate setup is shown in Figure 2.2.

![LONG®R3 IGF-I ELISA Kit example plate setup](Figure 2.2)

2.5 ELISA Procedure
Remove the LONG®R3 IGF-I ELISA plate and TMB (Reagent E) from 2 - 8°C storage and allow to equilibrate to room temperature for a minimum of 10 minutes. Ensure samples, standards and reagents are prepared before proceeding.

1. Wash the LONG®R3 IGF-I ELISA plate twice with 1X PBS buffer and discard all liquid from the wells. Remove excess liquid by inverting the plate on clean paper towels and tapping gently.
2. Use the same pipette tip to transfer 100 µL of the 40 ng/mL LONG®R3 IGF-I Reference Standard to wells A1-A3. Discard the pipette tip and replace with a new one for the next standard. Transfer remaining standards and the blank to the plate. Transfer 100 µL of each sample to the plate in triplicate and do the same for the standards. For best results, transfer standards and samples to the plate consecutively and without pausing.
3. Cover the plate and incubate at room temperature for 2 hours.
4. After incubation, wash the wells three times with PBS-Tween 20 Wash Solution and remove the liquid. Dry thoroughly by inverting plate on clean paper towels and tapping gently.
5. Prepare Detection Antibody Solution by adding 10 mL of the PBS-Tween 20 to a 15 mL conical tube and set aside. Take Reagent C vial of LONG®R3 IGF-I ELISA lyophilized Detection Antibody and add 1 mL of PBS-Tween 20. Transfer reconstituted Reagent C to 15 mL conical tube. Mix by inverting 10 times. Using a 12-channel pipette, add 100 µL of Detection Antibody Solution to all wells.
6. Cover the plate and incubate at room temperature for 1 hour.
7. After incubation, discard the Detection Antibody Solution and wash wells three times with PBS-Tween 20. Dry thoroughly by inverting plate on clean paper towels and tapping gently.
8. Prepare HRP-Streptavidin Solution by adding 12 mL of PBS-Tween 20 to a 15 mL tube. Add 12 µL of HRP-Streptavidin conjugate (Reagent D) and mix thoroughly by inverting 10 times. Using a 12-channel pipette, add 100 µL of HRP-Streptavidin Solution to all wells on the plate.
9. Cover the plate and incubate at room temperature for 30 minutes.
10. After incubation, discard conjugate solution. Wash wells twice with PBS-Tween 20.
11. Wash wells once more with 1X PBS. Dry thoroughly by inverting plate on clean paper towels and tapping gently.
12. Ensure that TMB- Peroxidase Substrate (Reagent E) is at room temperature before use. Using a 12-channel pipette, add 100 µL of Reagent E to all wells.
13. Cover the plate and incubate for 15 minutes. Stop the reaction by adding 100 µL of 4N sulfuric acid to all wells in the same order as the Reagent E. This will cause the solution to turn yellow.
14. Read the plate at 450 nm.

3. Calculation of Results
   1. Fit the standard curve to a 4-parameter fit. See Figure 3.1 for a typical standard curve.
   2. Calculate the concentration of LONG®R³ IGF- I in each Sample using the standard curve. Be sure to account for any dilution factors.

![Figure 3.1 Representative LONG®R³ IGF-I Standard Curve](image)

4. Frequently Asked Questions
   1. **Can I use this ELISA to quantitate IGF-I or Insulin as well as LONG®R³ IGF-I?**
      No, it is not possible using this assay. The antibodies used in Repligen’s LONG®R³ IGF-I ELISA do not interact with insulin and only have ~40% reactivity with IGF-I.
   2. **How can I store reconstituted Reference Standard (Reagent B)?**
      Reconstituted Reagent B can be stored for up to 1 month at 2-8°C.
   3. **How should I store samples to minimize adsorptive losses?**
      LONG®R³ IGF-I adsorbs to plastic and glass surfaces in the absence of other proteins or surfactants. Samples should be collected in low-binding tubes.
5. Troubleshooting

<table>
<thead>
<tr>
<th>Problem: There is no signal on the entire plate</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection antibody or Streptavidin-HRP may have been omitted or reagents added in wrong order.</td>
<td>Repeat assay and verify addition of all reagents in correct order.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Problem: There is very low signal on the entire plate</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection antibody or Streptavidin-HRP may have been added at incorrect dilutions.</td>
<td>Repeat assay verifying dilutions of reagents.</td>
<td></td>
</tr>
<tr>
<td>Plate reader may be set to the incorrect wavelength.</td>
<td>Repeat assay verifying 450 nm wavelength.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Problem: There is no signal from my samples</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples may not contain LONG®R³ IGF-I. ELISA will not accurately quantitate IGF-I or insulin. Samples may have been over-diluted.</td>
<td>Check sample preparation and dilution factors.</td>
<td></td>
</tr>
<tr>
<td>Samples may have adsorbed to collection tube.</td>
<td>Collect samples in low-binding tubes.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Problem: There is high well to well variability</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate washing may have been inconsistent.</td>
<td>Repeat assay verifying number of washes and check for washing of each well or column.</td>
<td></td>
</tr>
<tr>
<td>Pipetting may have been inaccurate.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Problem: There is high signal on the entire plate</th>
<th>Possible Cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate washing may have been insufficient.</td>
<td>Repeat assay verifying number of washes and check for washing of each well or column.</td>
<td></td>
</tr>
<tr>
<td>Detection antibody or Streptavidin-HRP may have been added at incorrect dilutions.</td>
<td>Repeat assay verifying dilutions of reagents.</td>
<td></td>
</tr>
<tr>
<td>Re-use of pipette tips, plate sealers or reagent reservoirs may have resulted in HRP contamination.</td>
<td>Use new plate sealers and reservoirs.</td>
<td></td>
</tr>
</tbody>
</table>
6. Safety Data Sheet

Reagent D – Component of LONG®R³ IGF-I Kit 9547

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

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

<table>
<thead>
<tr>
<th>CAS EC No Registration No</th>
<th>Component Name Synonyms</th>
<th>1272/2008 (CLP)</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>55965-84-9 -- --</td>
<td>5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone</td>
<td>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</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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, CO2, 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

Firefighters 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:


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

<table>
<thead>
<tr>
<th>Component Exposure Limits</th>
<th>5-Chloro-2-methyl-3(2H)-isothiazolone, mixture with 2-methyl-3(2H)-isothiazolone</th>
<th>55965-84-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria:</td>
<td>0.05 mg/m³ TWA [TMW ]</td>
<td>skin notation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin sensitizer</td>
</tr>
</tbody>
</table>

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 goggles 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).

Section 9 – Physical and Chemical Properties

9.1 Information on basic physical and chemical properties

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Colorless liquid</th>
<th>Physical State</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odor</td>
<td>Not available</td>
<td>Color</td>
<td>Colorless, clear to light yellow</td>
</tr>
<tr>
<td>Odor Threshold</td>
<td>Not available</td>
<td>pH</td>
<td>7</td>
</tr>
<tr>
<td>Melting Point</td>
<td>Not available</td>
<td>Boiling Point</td>
<td>Not available</td>
</tr>
<tr>
<td>Boiling Point Range</td>
<td>Not available</td>
<td>Freezing point</td>
<td>Not available</td>
</tr>
<tr>
<td>Evaporation Rate</td>
<td>Not available</td>
<td>Flammability (solid, gas)</td>
<td>Not available</td>
</tr>
<tr>
<td>Autoignition Temperature</td>
<td>Not available</td>
<td>Flash Point</td>
<td>Not available</td>
</tr>
<tr>
<td>Lower Explosive Limit</td>
<td>Not available</td>
<td>Decomposition temperature</td>
<td>Not available</td>
</tr>
<tr>
<td>Upper Explosive Limit</td>
<td>Not available</td>
<td>Vapor Pressure</td>
<td>Not available</td>
</tr>
<tr>
<td>Vapor Density (air=1)</td>
<td>Not available</td>
<td>Specific Gravity (water=1)</td>
<td>Not available</td>
</tr>
<tr>
<td>Water Solubility</td>
<td>(soluble)</td>
<td>Partition coefficient: n-octanol/water</td>
<td>Not available</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Not available</td>
<td>Solubility (Other)</td>
<td>Not available</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------</td>
<td>-------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Density</td>
<td>Not available</td>
<td>Physical Form</td>
<td>liquid</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>Not available</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<p>| | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Dermal</td>
<td>&gt; 2000 mg/kg</td>
</tr>
<tr>
<td>Oral</td>
<td>&gt; 2000 mg/kg</td>
</tr>
</tbody>
</table>

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

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

<table>
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</thead>
<tbody>
<tr>
<td>No</td>
<td>DSL</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

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); ENCS - European Inventory of Existing Commercial Chemical Substances; EU – European Commission; 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)
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.