PrepEase™ Quick MiniSpin Plasmid Kit

Product Number 78740, 10 preps
Product Number 78741, 50 preps
Product Number 78742, 250 preps

STORAGE
Store at room temperature (20–25°C).

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.
CONTENTS
Components .................................................................................................3
PrepEase™ Quick MiniSpin Plasmid Kit, 10 preps .........................................3
PrepEase™ Quick MiniSpin Plasmid Kit, 50 preps ........................................3
PrepEase™ Quick MiniSpin Plasmid Kit, 250 preps ......................................3
Storage and Preparation .............................................................................3
Materials Not Supplied ................................................................................4
Quality Control ............................................................................................4
Safety Warnings and Precautions ...............................................................4
Introduction ................................................................................................5
Overview ......................................................................................................5
Properties of PrepEase™ Membrane Filters ................................................6
Elution Procedures ......................................................................................6
Protocol .......................................................................................................7
Preparation of High-Copy Plasmid DNA ......................................................7
Supplementary Information ........................................................................8
Growing Bacterial Cultures .........................................................................8
Selection of Culture Media ..........................................................................8
Difficult-To-Lyse Strains ............................................................................9
Clarification of Lysates ...............................................................................9
Troubleshooting ..........................................................................................9
References ................................................................................................11
Related Products .........................................................................................12
Contact Information ...................................................................................13

COMPONENTS
Always keep buffer bottles tightly closed, especially if buffers are warmed
during preparation.

PrepEase™ Quick MiniSpin Plasmid Kits

<table>
<thead>
<tr>
<th></th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Buffer*</td>
<td>5 ml</td>
<td>15 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>A2 Buffer*</td>
<td>5 ml</td>
<td>15 ml</td>
<td>3 x 25 ml</td>
</tr>
<tr>
<td>A3 Buffer</td>
<td>5 ml</td>
<td>20 ml</td>
<td>100 ml</td>
</tr>
<tr>
<td>AQ Buffer (concentrate)</td>
<td>2 ml</td>
<td>7 ml</td>
<td>2 x 20 ml</td>
</tr>
<tr>
<td>AE Buffer</td>
<td>5 ml</td>
<td>15 ml</td>
<td>75 ml</td>
</tr>
<tr>
<td>RNase A* (lyophilized)</td>
<td>2 mg</td>
<td>6 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>PrepEase™ Quick MiniSpin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmid Columns</td>
<td>10</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>PrepEase™ Collecting Tubes (2 ml)</td>
<td>10</td>
<td>50</td>
<td>250</td>
</tr>
</tbody>
</table>

*See specific storage and preparation information below.

STORAGE AND PREPARATION
All kit components may be stored at room temperature (20-25°C).

RNase A Solution: Dissolve the lyophilized RNase A by adding 1 ml of A1 Buffer
directly into the bottle. Pipette up and down until the RNase A is dissolved
completely. Transfer the RNase A solution to the bottle containing the remaining
A1 Buffer and mix well. Write the date of RNase A addition onto the A1 Buffer
bottle. Store RNase A-A1 Buffer at 4°C.

<table>
<thead>
<tr>
<th></th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase A</td>
<td>2 mg</td>
<td>6 mg</td>
<td>30 mg</td>
</tr>
<tr>
<td>A1 Buffer</td>
<td>add 1 ml</td>
<td>add 1 ml</td>
<td>add 1 ml</td>
</tr>
</tbody>
</table>

Transfer RNase A solution to the bottle containing the remaining A1 Buffer and
mix well.

A2 Buffer contains SDS. It should be stored at room temperature (20-25°C)
since the SDS may precipitate at temperatures below 20°C. If precipitation
occurs, incubate the bottle for several minutes at about 30-40°C and mix well
until the precipitate is redissolved.
**AQ Buffer:** The AQ Buffer is supplied in concentrated form. Add the indicated amount of 96-100% ethanol to make complete AQ Buffer.

<table>
<thead>
<tr>
<th></th>
<th>10 preps</th>
<th>50 preps</th>
<th>250 preps</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQ Buffer (concentrate)</td>
<td>2 ml</td>
<td>2 x 7 ml</td>
<td>2 x 20 ml</td>
</tr>
<tr>
<td>Ethanol</td>
<td>add 8 ml</td>
<td>add 28 ml</td>
<td>add 80 ml</td>
</tr>
</tbody>
</table>

**(per A4 bottle)**

**MATERIALS NOT SUPPLIED**

96-100% Ethanol

1.5 ml microcentrifuge tubes

**QUALITY CONTROL**

PrepEase™ Quick MiniSpin Plasmid Kits contain PrepEase™ spin columns, RNase A and all the necessary buffers to purify high quality plasmid DNA. All PrepEase™ Quick MiniSpin Plasmid Columns are resistant to organic solvents such as alcohol, chloroform, and phenol and are free of contaminating DNase and RNase.

**SAFETY WARNINGS AND PRECAUTIONS**

**Warning:** For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. USB recommends that this product be used only by persons who have been trained in the principles of good laboratory practice. Wear suitable protective clothing such as laboratory coat, safety glasses, and gloves. Avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water (see ‘Material Safety Data Sheet’ for specific advice).

A3 Buffer contains guanidine hydrochloride. Proper safety precautions are recommended.

**INTRODUCTION**

**Overview**

The PrepEase™ Quick MiniSpin Plasmid Kit enables rapid, simple purification of plasmid DNA from small volumes of bacterial culture. The kit employs an alkaline lysis procedure and spin columns containing special patented anion-exchange membrane filters, which save time by eliminating extra wash and drying steps. Pelleted cells are resuspended (A1 Buffer), lysed (A2 Buffer), and neutralized in the presence of a chaotropic salt (A3 Buffer). The lysis step denatures both chromosomal and plasmid DNA. The neutralization step precipitates chromosomal DNA and other cellular components, which are then removed by centrifugation to yield a clarified lysate. The clarified lysate contains plasmid DNA and contaminating proteins and other soluble cellular components. The clarified lysate is passed through a PrepEase™ Quick MiniSpin Column by centrifugation, resulting in reversible binding of the plasmid DNA to the anion-exchange membrane filter due to the presence of the chaotropic salt. The membrane filter is washed (AQ Buffer) to remove non-DNA contaminants. This single wash, without need for any subsequent drying step, is sufficient. Finally, plasmid DNA is eluted from the membrane filter (AE Buffer). The eluted plasmid DNA is suitable for use in many common applications, such as automated fluorescent DNA sequencing, PCR, and enzymatic manipulations.

The PrepEase™ Quick MiniSpin Plasmid Kit protocol is designed for preparation of plasmids ranging up to 15 kb in size, and for high-copy plasmids in particular. Simple modifications of the protocol enable use of a wide variety of bacterial strains and growth media, and optimization of elution procedures. For preparation of low-copy plasmids, try the USB PrepEase™ MiniSpin Plasmid Kit (PN 78735).

**Kit Specifications**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PrepEase™ Quick MiniSpin Plasmid Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture volume (high-copy)</td>
<td>1-3 ml</td>
</tr>
<tr>
<td>Elution volume</td>
<td>50 µl</td>
</tr>
<tr>
<td>Binding capacity</td>
<td>15 µg</td>
</tr>
<tr>
<td>Plasmid size</td>
<td>up to 15 kb</td>
</tr>
<tr>
<td>Time per prep</td>
<td>15 min</td>
</tr>
<tr>
<td>Column type</td>
<td>mini-spin column</td>
</tr>
</tbody>
</table>
Properties of PrepEase™ Membrane Filters

A specially activated silica membrane filter used in the spin-column format is the key to rapid, simple preparation of plasmid DNA with the PrepEase™ Quick MiniSpin Plasmid Kit. The membrane filter is designed to selectively bind negatively charged plasmid DNA, allowing easy separation from contaminating proteins and other cellular components. Furthermore, the filter is specially treated to enable removal of the non-DNA contaminants in a single wash step, without need for subsequent drying. This combination of properties provides unparalleled ease and speed in preparation of plasmid DNA by the spin column technique.

Elution Procedures

The PrepEase™ Quick MiniSpin Plasmid Kit is designed to provide flexibility in the elution step, regarding yield, concentration, and elution reagents. The standard protocol for elution with AE Buffer at room temperature provides a yield of approximately 70-90%. The yield and/or concentration can be increased with the following approaches, each of which requires use of AE Buffer at 70°C.

**High yield:** Perform two elution steps, each with 50 µl AE Buffer. Yield increases to approximately 90-100% but with a corresponding decrease in plasmid concentration.

**High concentration:** Perform one elution step using only 30 µl of AE Buffer. Concentration increases to approximately 130% but with a corresponding decrease in yield (maximum yield of 80%).

**High yield and concentration:** Perform two elution steps, each with 25 µl AE Buffer. Yield increases to approximately 85%-100% with no decrease in concentration.

AE Buffer can be replaced by other slightly alkaline, weakly buffered reagents such as TE or 10mM Tris-HCl, pH 8.0. Dilute Tris is particularly recommended for elution of plasmid DNA that will be used in sequencing reactions. Water may also be used for elution, but its pH should be checked and adjusted to pH 8-8.5 prior to use in elution.

PROTOCOL:

**Preparation of High-Copy Plasmid DNA**

1. **Prepare an overnight culture**
   Begin with an isolated bacterial colony from a fresh plate and inoculate an appropriate volume of LB medium (1-5 ml) that includes appropriate antibiotics. Incubate overnight with shaking (12-16 hrs). Refer to the Supplementary Information included in this protocol for advice on growing bacterial cultures.

2. **Harvest bacterial cells**
   Harvest bacteria from 1-3 ml LB culture by transferring aliquots of culture to a 1.5 ml microcentrifuge tube, conducting centrifugation at 11,000 x g for 30 sec, and carefully discarding the supernatant. Repeat as necessary to obtain a cell pellet from the desired culture volume in the microcentrifuge tube.
   
   This and all subsequent steps are carried out at room temperature (20-25°C), except as otherwise noted.

3. **Cell Lysis and Neutralization**
   a. Resuspend the pellet of bacterial cells in 250 µl RNase A-A1 Buffer by vigorous vortexing. Refer to the Supplementary Information for advice on difficult-to-lyse strains.
   b. Lyse the suspension by adding 250 µl A2 Buffer. Mix gently by inverting the tube 6-8 times. Incubate the resulting lysate for 2–3 min (max 5 min) at room temperature. Do not vortex, as this will release contaminating chromosomal DNA from cellular debris into the suspension.
   c. Neutralize the lysate by adding 300 µl A3 Buffer. Immediately gently mix by inverting the tube 6-8 times until a homogeneous suspension containing an off-white flocculate is formed.
   
   Note that aliquots from this and all subsequent steps may be retained for troubleshooting purposes.

4. **Clarify the lysate**
   Clarify the lysate by centrifugation at 11,000 x g for 5 min. This step can be carried out at room temperature for convenience, or at 4°C to promote more effective precipitation of cell debris.

5. **Bind plasmid to column**
   Place a PrepEase™ Quick MiniSpin Column in a 2 ml collecting tube. Add clarified lysate onto the column. Conduct centrifugation at 11,000 x g for 1 min. Discard the flow-through.
6. Wash and dry the column
Place the PrepEase™ Quick MiniSpin Column back into the 2 ml collecting tube. Add 450 µl AQ Buffer (complete with ethanol). Conduct centrifugation at 11,000 x g for 4 min. Discard the flow-through. Note that this step also accomplishes drying of the column, thus preventing carryover of ethanol supernatant into subsequent steps.

7. Elution
Place the PrepEase™ Quick MiniSpin Column into a 1.5 ml microcentrifuge tube. Add 50 µl AE Buffer. Incubate 1 min. Conduct centrifugation at 11,000 x g for 1 min. Flow-through contains the eluted plasmid. See Elution Procedures in Introduction for optimization of yield and/or concentration and alternative elution reagents.

SUPPLEMENTARY INFORMATION
Yield and quality of plasmid DNA depends on many factors. The type of growth media and antibiotics, bacterial host, and plasmid type, size, and copy number all contribute to a successful plasmid prep.

Growing Bacterial Cultures
Culture volumes given in the protocol refer to typical Escherichia coli strains cultivated overnight to an OD 600 of 3.0-6.0. Optimal culture densities and sample volumes may vary depending on the bacterial host and can be adjusted accordingly.

Important: Do not overload the PrepEase™ Quick MiniSpin Columns with bacterial material. Both the yield and quality of plasmid will decrease.

Selection of Culture Media
LB (Luria-Bertani) medium is recommended for use with the PrepEase™ Quick MiniSpin Plasmid Kit. The cultivation of cells at 37°C with constant shaking (200 - 250 rpm) is recommended. Alternatively, rich media like 2X YT (Yeast/ Tryptone) or TB (Terrific Broth) may be used. However, bacteria grow faster and reach the stationary phase much earlier in 2X YT or TB than in LB medium (≤ 12 hrs). This may lead to a higher percentage of dead or starving cells when starting the preparation. Thus, the plasmid DNA from overgrown cultures may be partially degraded or may be contaminated with chromosomal DNA. When using rich media, shorten growth times appropriately (i.e., ≤ 12 hrs).

Antibiotic Selection

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Stock Solution</th>
<th>Storage</th>
<th>Working Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>50 mg/ml in H₂O</td>
<td>-20°C</td>
<td>50 - 100 µg/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>34 mg/ml in EtOH</td>
<td>-20°C</td>
<td>25 - 170 µg/ml</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>10 mg/ml in H₂O</td>
<td>-20°C</td>
<td>10 - 50 µg/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>10 mg/ml in H₂O</td>
<td>-20°C</td>
<td>10 - 50 µg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>5 mg/ml in EtOH</td>
<td>-20°C</td>
<td>10 - 50 µg/ml</td>
</tr>
</tbody>
</table>

Difficult-To-Lyse Strains
Some strains of bacteria may be difficult to lyse. To prepare plasmid DNA from these strains, resuspend the pellet in RNase A-A1 Buffer containing lysozyme at a 2 mg/ml final concentration. Incubate at 37°C for 30 minutes and then continue the protocol with the addition of A2 Buffer.

Clarification of Lysates
Optimal centrifugation times for clarification of lysates may vary depending on choice of growth medium and culture volume. Centrifugation times of five to ten minutes typically work well.

TROUBLESHOOTING
In general, if reduced yield or purity is an issue, check each purification step to determine where the problem occurred. First, check that the bacterial cell culture grew sufficiently (OD 600 = 3.0-6.0) in the presence of an appropriate selective antibiotic. Second, check the aliquots kept from the cleared lysate, flow-through, washing step(s), and the eluate by agarose gel electrophoresis. The aliquots may need to be concentrated by ethanol precipitation in order to have a sufficient amount of sample to analyze on the gel.

Problem Possible Causes and Solutions
Incomplete lysis of bacterial cells
1. Cell pellet not properly resuspended
   Cell pellet must be completely resuspended prior to lysis. No cell clumps should be visible before addition of A2 Buffer.
2. SDS precipitation in A2 Buffer
   SDS in A2 Buffer may precipitate upon storage. If a precipitate has formed, incubate A2 Buffer at 30–40°C for 5 min and mix well.
3. Excessive amounts of bacteria used
   LB growth medium is optimal. Use of very rich media like TB (Terrific Broth) may result in culture cell densities that are too high.
Low plasmid yield

1. Incomplete lysis of bacterial cells
   See “Possible Causes and Solutions” above.

2. Suboptimal precipitation of SDS and cell debris
   Consider clarifying cell lysate by centrifugation at 4°C rather than room temperature. Precipitation of SDS and cell debris will be slightly more effective at the lower temperature.

3. Insufficient amounts of antibiotic used during cultivation
   Cells carrying the plasmid of interest may become overgrown by cells lacking the plasmid when inadequate levels of the appropriate antibiotic are used. Prepare fresh stocks of antibiotics and use the appropriate amounts in all solid and liquid media.

4. Bacteria cultivated for an excessive length of time
   Do not incubate cultures for longer than 16 hrs at 37°C under shaking. LB medium is optimal. When using very rich media like TB (Terrific Broth), cultivation time should be reduced to less than 12 hrs.

5. Suboptimal elution conditions
   If reagent other than AE Buffer is used for elution, the reagent must be slightly alkaline. If nuclease-free water is used, check pH. Elution efficiencies drop drastically at pH less than 7.

6. Low-copy plasmid
   For preparation of low-copy plasmids, use USB PrepEase™ MiniSpin Plasmid Kit (PN 78735, 78736, and 78737).

No plasmid yield

1. Ethanol not added to AQ Buffer
   Make complete AQ Buffer by adding the appropriate volume of ethanol before use.

2. Nuclease-rich host strains used
   When working with nuclease-rich strains, keep plasmid preparations on ice or frozen in order to avoid DNA degradation.

3. Inappropriate storage of plasmid DNA
   Quantify plasmid DNA directly after preparation, e.g. by agarose gel electrophoresis or A<sub>260</sub>. Store plasmid DNA in AE Buffer or TE at +4°C or in water at -20°C.

Poor plasmid quality

1. Nicked plasmid DNA
   Never incubate cell suspension in alkaline lysis A2 Buffer for longer than 5 min.

2. Genomic DNA contamination
   Avoid vortexing or vigorously mixing lysate after addition of A2 Buffer.

3. Smearred plasmid bands on agarose gel
   See information about nuclease-rich host strains, above.

Suboptimal performance of plasmid DNA in enzymatic reactions

1. Carryover of ethanol
   Be certain to carry out centrifugation for at least 4 min after washing to completely remove AQ Buffer.

2. Elution of plasmid DNA with TE buffer
   EDTA may inhibit sequencing reactions. Repurify plasmid DNA and elute with AE Buffer or water. Alternatively, the eluted plasmid DNA can be precipitated with ethanol and then redissolved in AE Buffer or water.

3. Insufficient amount of DNA used for sequencing reaction
   Quantify DNA by agarose gel electrophoresis or A<sub>260</sub> prior to setting up sequencing reactions.

4. Excessive amounts of bacteria used
   Do not use more than 3 ml of a saturated E. coli culture if preparing plasmid DNA for automated fluorescent DNA sequencing.

If problems persist please contact USB Technical Support for assistance at (800) 321-9322 or techsupport@usbweb.com. For technical support outside the U.S., please visit our website for up-to-date contact information on the USB product distributor within your area.

REFERENCES

### RELATED PRODUCTS

#### Ultrapure Reagents

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose - Separation ≥ 500 bp, Genetic Performance Certified™</td>
<td>Gel electrophoresis</td>
<td>25 gm 100 gm 250 gm 500 gm</td>
<td>75817</td>
</tr>
<tr>
<td>Agarose – LE</td>
<td>Gel electrophoresis</td>
<td>25 gm 100 gm 250 gm 500 gm</td>
<td>32802</td>
</tr>
<tr>
<td>LB Broth, Ready-Made Powder</td>
<td>Bacterial culture</td>
<td>250 gm 1 kg</td>
<td>75852</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Bacterial cell lysis</td>
<td>1 gm 5 gm 10 gm 25 gm 100 gm</td>
<td>18645</td>
</tr>
<tr>
<td>RapidRun™ Agarose Buffer, 20X Solution</td>
<td>Gel electrophoresis</td>
<td>1 L 5 L</td>
<td>77523</td>
</tr>
<tr>
<td>TBE Buffer, 5X Solution</td>
<td>Gel electrophoresis</td>
<td>1 L 5 L</td>
<td>75891</td>
</tr>
<tr>
<td>TBE Buffer, 10X Ready-Mixed Powder</td>
<td>Gel electrophoresis</td>
<td>6 x 200 ml</td>
<td>70454</td>
</tr>
<tr>
<td>TAE Buffer, 10X Solution</td>
<td>Gel electrophoresis</td>
<td>1 L 5 L</td>
<td>75904</td>
</tr>
<tr>
<td>TE Buffer, 1X Solution</td>
<td>Storage of DNA</td>
<td>10 x 1 ml 100 ml 500 ml</td>
<td>75893</td>
</tr>
<tr>
<td>Water, Nuclease-Free</td>
<td>Resuspending plasmid</td>
<td>10 x 1 ml 100 ml 500 ml 1 L 5 L</td>
<td>71786</td>
</tr>
</tbody>
</table>

#### Ultrapure Antibiotics

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin, Sodium Salt</td>
<td>Cell culture</td>
<td>5 gm 25 gm 100 gm</td>
<td>11259</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Cell culture</td>
<td>5 gm 25 gm 100 gm</td>
<td>23660</td>
</tr>
<tr>
<td>Kanamycin Sulfate</td>
<td>Cell culture</td>
<td>5 gm 25 gm</td>
<td>17924</td>
</tr>
</tbody>
</table>

#### USB CORPORATION

USA
Cleveland, Ohio
(800) 321-9322
www.usbweb.com

USB Europe GmbH
Staufen, Germany
+49(0)76 33 - 933 400
www.usbweb.com

USB products distributed outside the USA:
Please visit the USB website at www.usbweb.com for up-to-date contact information within your area.
Material Safety Data Sheet
Revision: 01/24/2006

Hazard information is provided for compliance with both the
UK Chemicals (Hazard Information and Packaging) (CHIP)
Regulations and the US Hazard Communication Standard (HCS)

IDENTIFICATION OF THE
SUBSTANCE/PREPARATION
AND COMPANY

PREP-EASE™ Quick MiniSpin Plasmid Kit
PRODUCT CODE: 78740 / 78741 / 78742

SUPPLIER:
USB Corporation
26111 Miles Road, Cleveland, Ohio 44128
Phone: (216) 765-5000
Outside USA & Canada: 703-527-3887

EMERGENCY CONTACT:
Chemtrec: (800) 424-9300

Please visit our website at www.usbweb.com for contact
information on USB product distributors within your area.

COMPOSITION/HAZARDOUS COMPONENTS

<table>
<thead>
<tr>
<th>HAZARD</th>
<th>CAS NO.</th>
<th>%WT</th>
<th>TLV</th>
<th>CHIP R &amp; S Phrases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanidine Hydrochloride (A3 Buffer)</td>
<td>50-01-1</td>
<td>~30%</td>
<td>—</td>
<td>R22 Harmful if swallowed. R36/38 Irritating to eyes and skin. S23 Do not breathe vapour.</td>
</tr>
<tr>
<td>Sodium Dodecyl Sulfate (SDS) (A2 Buffer)</td>
<td>151-21-3</td>
<td>~1.0%</td>
<td>—</td>
<td>For A2 Buffer: R22 Harmful if swallowed. R36/37/38 Irritating to eyes, respiratory system and skin. S24/25 Avoid contact with skin and eyes. S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.</td>
</tr>
<tr>
<td>Sodium Hydroxide (A2 Buffer)</td>
<td>1310-73-2</td>
<td>&lt;1%</td>
<td>See “Regulatory Information” Section</td>
<td></td>
</tr>
</tbody>
</table>

HAZARDS IDENTIFICATION

CHIP: Harmful (Guanidine Hydrochloride and SDS); Irritant (Sodium Hydroxide)
HCS: Toxic (Guanidine Hydrochloride); Irritant (SDS and Sodium Hydroxide)

FIRST-AID MEASURES

EYES: Flush with water for 15 minutes. Seek medical advice if irritation persists.
SKIN: Flush with water, then wash thoroughly with soap and water. Remove contaminated clothing and wash before reuse. Seek medical attention if irritation persists.

INHALATION: Remove the victim from exposure and move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Keep victim quiet and warm. Seek immediate medical attention.

INGESTION: Drink water and seek immediate medical attention. Avoid alcoholic beverages. Never give anything by mouth to an unconscious person.

FIRE-FIGHTING INFORMATION

Use media suitable to extinguish the supporting or surrounding fire. Wear NIOSH (or equivalent) approved self contained breathing apparatus. For small fires only: use carbon dioxide, dry powder or foam. Emits toxic fumes under fire conditions. Flash Point = 212°F (Closed cup for SDS).

ACCIDENTAL RELEASE MEASURES

Eliminate all sources of ignition. Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. Use non-sparking tools. Absorb with sand or vermiculite. Collect in a manner that does not create dust and place in a suitable waste container. Avoid contact of material with skin or eyes. Use adequate ventilation.

HANDLING AND STORAGE

Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. Avoid contact of material with skin or eyes. Use adequate ventilation. Avoid heat, sparks and open flame. Store ambient away from incompatible materials.

PERSONAL PROTECTION

Wear appropriate personal protective equipment and clothing including lab coat, safety goggles, gloves and NIOSH-approved respirator. A qualified industrial hygienist should evaluate the need for respiratory protection. Use respiratory protection approved by NIOSH (or equivalent) and appropriate to the hazard. Avoid contact of material with skin or eyes. Mechanical ventilation or local exhaust as needed to control exposure to dust, vapors or mists. Access to a safety shower and eye-wash.

PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Kit containing vials of solution (SDS may precipitate)
Boiling Point: No data available
Vapor Pressure: No data available
Vapor Density: No data available
Solubility (Water): Soluble
Specific Gravity: No data available
Percent Volatile: No data available
Evaporation Rate: No data available
Chemical Formula: Kit
STABILITY AND REACTIVITY

Product is stable. Avoid heat and acidic conditions. **For A3 Buffer:** Hazardous decomposition products include hydrogen chloride gas and oxides of nitrogen and carbon. Incompatible with strong oxidizing agents. Hazardous polymerization will not occur. **For A2 Buffer:** Hazardous decomposition products include sulfur, carbon and sodium oxides. Incompatible with strong oxidizing agents, metals, acids, aluminum, tin and zinc. Hazardous polymerization will not occur.

TOXICOLOGICAL INFORMATION

**EFFECTS OF OVEREXPOSURE:**

**FOR A3 BUFFER (Guanidine Hydrochloride):**
- **EYES:** Vapors may cause irritation or burning upon contact.
- **SKIN:** May be harmful if absorbed through the skin. Causes severe irritation to skin and hypersensitive individuals may experience an allergic reaction.
- **INHALATION:** May be harmful if inhaled. May cause irritation to mucous membranes and upper respiratory tract.
- **INGESTION:** Harmful if swallowed. Chronic ingestion or excessive dosage may cause gastrointestinal tract irritation with nausea, vomiting and diarrhea. May cause central nervous system disorders.

**TARGET ORGANS:** Bone Marrow and Nerves.

**FOR A2 BUFFER (SDS and Sodium Hydroxide):**
- **EYES:** Contact may cause severe irritation.
- **SKIN:** Contact may cause irritation. May cause skin sensitization, an allergic reaction, which becomes evident upon re-exposure to this material.
- **INHALATION:** May be harmful if inhaled. May cause allergic respiratory reaction. May cause irritation to mucous membranes and upper respiratory tract.
- **INGESTION:** Harmful if swallowed. Chronic ingestion or excessive dosage may cause irritation of the gastrointestinal tract with nausea, vomiting and diarrhea.

**ADDITIONAL INFORMATION:**

Only select RTECS information is provided here. Please see actual RTECS entries for complete information.

Irritation, mutation and toxicity data for Guanidine Hydrochloride listed in RTECS under MF4300000.

Irritation data: Skin Rabbit 500 mg/24H = Severe. Eye Rabbit 81400 ug = Moderate.

Toxicity data: Oral Rat LD50 = 475 mg/kg. Toxic effects may include altered sleep time (including change in righting reflex), excitement, hypermotility and diarrhea.

Reproductive effects, irritation, mutation and toxicity data for SDS listed in RTECS under WT1050000.

Irritation data: Skin Rabbit 50 mg/24H = Severe (1971). Eye Rabbit 100 mg/24H = Moderate (1972).

Toxicity data: Oral Rat LD50 = 1288 mg/kg (1967). Inhalation Rat LC50 = >3900 mg/m3/1H (1971).

Reproductive data: Effects on embryo or fetus included fetotoxicity (except death, e.g. stunted fetus).

Irritation, mutation and toxicity data for Sodium Hydroxide listed in RTECS under WB4900000.

Irritation data: Skin Rabbit 500 mg/24H = Severe (1972). Eye Rabbit 1 mg/24H = Severe (1964).

Toxicity data: Intraperitoneal Mouse LD50 = 40 mg/kg (1963).

Definition(s): RTECS = Registry of Toxic Effects of Chemical Substances.

OSHA = Occupational Safety and Health Administration.

ACGIH = American Conference of Governmental Industrial Hygienists.

ECOLOGICAL INFORMATION

No information available.

DISPOSAL CONSIDERATIONS

Dispose of material in accordance with applicable local, state, and federal regulations.

TRANSPORTATION INFORMATION

US DOT / IATA: No applicable information.

REGULATORY INFORMATION

RCRA - No applicable information.

SARA 302 - No applicable information.

SARA 313 - No applicable information.

SARA 311/312 - acute (Guanidine Hydrochloride).

EPA TSCA Section 8(b) - All listed components: Chemical Inventory.

Exposure Limits - For Sodium Hydroxide: ACGIH TLV-CL 2 mg/m3.

OSHA PEL (Gen Indu): 8H TWA 2 mg/m3.

California Proposition 65 - No applicable information.

This data sheet is based upon information believed to be reliable. The Company makes no statement or warranty as to the accuracy or completeness of the information contained herein which is offered for your consideration, investigation and verification. Any use of the information contained in this data sheet must be determined by the user to be in accordance with appropriate applicable regulations.