USB® PrepEase® Yeast Plasmid Isolation Kit

Product number 79220, 50 reactions

Storage
Store at -20°C (non-frost-free freezer).

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.
Product description
The USB PrepEase Yeast Plasmid Isolation Kit is a miniprep kit for yeast cells. It is designed for the isolation of 2 μ plasmids from yeast patches on plates or from yeast grown in liquid culture. The isolated plasmid DNA can be used for PCR amplification, bacterial transformation and DNA sequencing. The protocol eliminates the need for phenol/chloroform extraction, glass bead disruption or column elution. This kit may also be used to obtain spheroplasts for other experimental uses.

Storage
Important: This kit and all enclosed reagents should be stored at -20°C (non frost-free freezer). Keep Enzyme Solution at -20°C until ready to use. Allow remaining reagents to thaw at room temperature but do not heat. Place thawed Spheroplast Solution on ice and leave Lysis Solution and Precipitation Solution at room temperature. Before use it is important to gently vortex each reagent and briefly centrifuge to collect at the bottom of the tube. Perform all reactions at room temperature unless stated otherwise.

Quality control
All lots of reagents are functionally tested on 2 μ plasmid isolated from both a single yeast patch on a plate and from yeast grown in liquid culture (typical volume is 1.5 ml using yeast two-hybrid transformants). The isolated plasmid DNA is tested by sequencing with the Thermo Sequenase™ Dye Primer Manual Cycle Sequencing Kit (PN 79260) and TAMRA-labeled pGAD424 forward primer.

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.
Caution: All chemicals should be considered as potentially hazardous. We, therefore, recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing, such as lab coat, safety glasses, and gloves. Care should be taken to avoid contact with skin and eyes. In the case of contact with skin or eyes, wash immediately with water. See MSDS (Material Safety Data Sheet) for specific advice.
Components of the kit
Spheroplast Solution
Enzyme Solution
Lysis Solution
Precipitation Solution

Materials not supplied
100% Isopropanol
70% Ethanol
5% SDS

Protocol

1. Prepare yeast culture
   Liquid culture: Pick a colony and transfer to 1.5 ml of drop-out medium (to maintain selection for the plasmid) in a 17 x 100 mm sterile, polystyrene round-bottom tube. Shake overnight or longer at 30°C to reach a stationary phase.
   For yeast patches: With a pipet tip (200 μl) transfer a yeast patch from a plate into a 1.5 ml microcentrifuge tube. Continue with step 3 below.

2. Harvest yeast
   a. Transfer liquid culture to a 1.5 ml microcentrifuge tube and centrifuge at 6000 x g (7,500 rpm in a standard centrifuge) for 2 – 3 minutes to pellet the cells. Remove supernatant.
   b. Wash cells with 1 ml dH2O. Lightly vortex to resuspend cell pellet.
   c. Centrifuge at 6000 x g (7,500 rpm) for 2 – 3 minutes. Remove supernatant.

3. Yeast cell lysis
   a. Gently resuspend cell pellet in 100 μl Spheroplast Solution.
   b. Add 4 μl Enzyme Solution to the resuspended yeast cells. Mix well and incubate at 37°C for 1/2 to 2 hours. The optimal incubation time must be determined empirically and depends on the yeast strain, growth stage and culture condition. Occasionally tap the tube gently to disperse cells that collect at the bottom, being careful not to rupture the spheroplasts. The spheroplasts should appear as a sticky mass at the bottom of the microcentrifuge tube. Spheroplast formation can be verified by SDS lysis of the spheroplasts.
   c. Pipet 3 μl of the upper supernatant onto a glass slide and, using an inverted microscope, observe rupture of the spheroplasts upon addition of 3 μl of 5% SDS.
   d. Centrifuge at 6000 x g (7,500 rpm) for 4 minutes. Remove the supernatant very carefully.
   e. Add 150 μl Lysis Solution to the spheroplast pellet but do not resuspend. Instead, using the tip, grasp and transfer the pellet to a fresh 0.5 ml microcentrifuge tube. Transfer the remaining lysis buffer. Resuspend pellet.
   f. Incubate at 95°C for 10 minutes and chill on ice. It is convenient to do the incubation in a thermal cycler.

4. Clarify the lysate
   a. Add 50 μl of Precipitation Solution. Mix gently by inverting the tube 3 – 5 times. A white fluffy precipitate should be visible. Incubate on ice for 10 – 20 minutes.
   b. Centrifuge at 6000 x g (7,500 rpm) for 4 minutes.
   c. Discard the supernatant carefully to a fresh 0.5 ml microcentrifuge tube. Resuspend pellet.

5. Precipitate the yeast plasmid
   a. To the supernatant, add an equal volume of chilled 100% isopropanol. Mix by inversion and incubate the tube at -80°C for at least 1 hour.
   b. Centrifuge at 20,000 x g (14,000 rpm) for 20 minutes at 4°C. Remove supernatant. Care should be taken not to disturb the pellet. At this point the pellet should be ~0.5 to 1 mm in size and may appear translucent.
   c. Wash the pellet once with an equal volume of 70% ethanol. Centrifuge at 20,000 x g (14,000 rpm) for 5 minutes.

6. Resuspend the yeast plasmid
   a. Dry the pellet. If a vacuum centrifuge is utilized, do not over-dry the pellet.
   b. Resuspend the pellet in 13 μl of dH2O. Save 1 μl for future analysis and use remaining sample for PCR, transformation, or DNA sequencing.
# Related products

## Kits

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Pack size</th>
<th>Product number</th>
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<tbody>
<tr>
<td>Thermo Sequenase™ Dye Primer Manual Cycle Sequencing Kit</td>
<td>For manual cycle sequencing (fluorescent or radiolabeled primers)</td>
<td>50 rctns</td>
<td>79260</td>
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## PCR reagents

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<td>Taq PCR Master Mix (2X)</td>
<td>PCR</td>
<td>100 rctns</td>
<td>71162</td>
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<tr>
<td>HotStart-IT® Taq Master Mix (2X)</td>
<td>PCR</td>
<td>25 rctns</td>
<td>71196</td>
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<td>VeriQuest™ SYBR® Green qPCR Master Mix (2X) (50 µl/rctn)</td>
<td>qPCR</td>
<td>40 rctns</td>
<td>75600</td>
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<td>VeriQuest SYBR Green qPCR Master Mix with Fluorescein (2X) (50 µl/rctn)</td>
<td>qPCR</td>
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<tr>
<td>VeriQuest Probe qPCR Master Mix (2X) (50 µl/rctn)</td>
<td>qPCR</td>
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<td>75650</td>
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<tr>
<td>VeriQuest Probe qPCR Master Mix, No Reference Dye (2X) (50 µl/rctn)</td>
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<td>40 rctns</td>
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<tr>
<td>VeriQuest Fast SYBR Green qPCR Master Mix (2X) (20 µl/rctn)</td>
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<td>100 rctns</td>
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<td>VeriQuest Fast SYBR Green qPCR Master Mix with Fluorescein (2X) (20 µl/rctn)</td>
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## Reagents

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<td>SDS, 10% Solution</td>
<td>Cell lysis</td>
<td>100 ml</td>
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<tr>
<td>YPD Agar</td>
<td>Yeast propagation</td>
<td>250 gm</td>
<td>75857</td>
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<tr>
<td>YPD Broth</td>
<td>Yeast propagation</td>
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## Affymetrix, Inc.

usb.affymetrix.com

USA
Cleveland, Ohio
(888) 362-2447 | (216) 765-5000

Europe
High Wycombe, United Kingdom
+44 (0)1628 55 2600

USB products distributed outside the USA:
Please visit our website at usb.affymetrix.com for up-to-date contact information within your area.
Material Safety Data Sheet

Revision: 08/30/2007

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 PRODUCT

SUBSTANCE/PREPARATION: USB® PrepEase® Yeast Plasmid Isolation Kit

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

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

EMERGENCY CONTACT:
USB Products – Affymetrix, Inc. Chemtrec (800) 424-9300
26111 Miles Road, Cleveland, Ohio 44128 Phone: (216) 765-5000
Outside USA & Canada (703) 527-3887

COMPOSITION/HAZARDOUS COMPONENTS

<table>
<thead>
<tr>
<th>COMPONENT</th>
<th>CAS NO.</th>
<th>% WT</th>
<th>TLV</th>
<th>CHIP R &amp; S PHRASES</th>
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</thead>
<tbody>
<tr>
<td>Glycerol in 79226</td>
<td>56-81-5</td>
<td>~50.0% OSHA TWA:</td>
<td>R:36/37/38 Irritating to eyes, respiratory system and skin.</td>
<td></td>
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<tr>
<td>2-Mercaptoethanol in 79227</td>
<td>60-24-2</td>
<td>1.0%</td>
<td>R:22 Harmful if swallowed. R:24 Toxic in contact with skin. R:34 Causes burns. R:51/53 Toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment. S:61 Avoid release to the environment. Refer to special instructions/safety data sheet.</td>
<td></td>
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<tr>
<td>Potassium Acetate in 79229</td>
<td>127-08-2</td>
<td>~78.5%</td>
<td>R:36/37/38 Irritating to eyes, respiratory system and skin.</td>
<td></td>
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<tr>
<td>For all kit components:</td>
<td></td>
<td></td>
<td></td>
<td>S:26 In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. S:36/37/39 Wear suitable protective clothing, gloves and ey/face protection.</td>
</tr>
</tbody>
</table>

HAZARDS IDENTIFICATION

CHIP
2-Mercaptoethanol: Toxic, Dangerous for the Environment; Glycerol and Potassium Acetate: Irritant

HCS
2-Mercaptoethanol: Toxic; Glycerol and Potassium Acetate: Irritant

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. For 2-Mercaptoethanol: Combustible Liquid. For Glycerol: Contact with strong oxidizing agents may produce an explosion. Explosion limits: For 2-Mercaptoethanol - Lower: 1.1 Upper: 2.7; For Glycerol - Lower: 1.1 Upper: Not available.
Flash Point: For 2-Mercaptoethanol = 74°C; For Glycerol = 193°C.
Autoignition Temperature: For 2-Mercaptoethanol = 295°C; For Glycerol = 400°C.

ACCIDENTAL RELEASE MEASURES

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NIOSH-approved respirator. Eliminate all sources of ignition. 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 glasses, gloves and NIOSH-approved respirator. Avoid contact of material with skin or eyes. Use adequate ventilation. Store in a cool, dry, well-ventilated area away from incompatible substances. For 2-Mercaptoethanol: Moisture sensitive. Keep away from heat, sparks and flame. For Potassium Acetate: Hygroscopic.

PERSONAL PROTECTION

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, 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 separate vials. Evaporation Rate: No data available.
Boiling Point: For Glycerol = 288°C; 2-Mercaptoethanol = 157°C. Specific Volatile: No data available.
Vapor Density: For 2-Mercaptoethanol = 2.69.
Vapor Pressure: For Glycerol = .0025 mm Hg@5; 2-Mercaptoethanol = 1 mm Hg@ 20°C.
Melting Point: For Glycerol = 20°F; For 2-Mercaptoethanol = -50°C; For Potassium Acetate = 292°C.

STABILITY AND REACTIVITY

Product is stable under normal conditions. Hazardous polymerization will not occur. Hazardous decomposition products include carbon oxides, oxides of sulfur and irritating and toxic fumes and gases. Incompatibilities: For 2-Mercaptoethanol avoid strong oxidizers, calcium hypochlorite, metals, strong acids, caustics, aliphatic amines and isocyanates; For Potassium Acetate avoid moisture; For Glycerol avoid strong oxidizing agents including mixtures with hydrogen peroxide, potassium permanganate, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide. Contact with Sodium Hypochlorite and Hypochlorous Acid may cause an explosion.

CONDITIONS TO AVOID:

For 2-Mercaptoethanol avoid high temperatures, ignition sources and excess heat; For Potassium Acetate avoid high temperatures, ignition sources and excess heat. 

TOXICOLOGICAL INFORMATION

EFFECTS OF OVEREXPOSURE: The toxicological properties of the PrepEase® Yeast Plasmid Isolation Kit have not been thoroughly investigated. Handle according to the precautions for the most hazardous component.

FOR GLYCEROL:
Oral Rat LD50 = 12600 mg/kg. EYES: Contact may cause irritation and slight corneal injury. SKIN: Prolonged contact may cause irritation and/or allergic reaction. INHALATION: No known toxicity, but excessive fumes may cause irritation if inhaled. INGESTION: May cause irritation of gastrointestinal tract and diarrhea. Irritation, mutation, reproductive effects and toxicity data for Glycerol is listed in RTECS under MA8050000.

FOR 2-MERCAPTOETHANOL:
Oral Rat LD50 = 244 mg/kg. Inhalation Rat LCLo = 250 ppm/8H. EYES: May cause severe eye irritation. May result in corneal injury. SKIN: May be fatal if absorbed through the skin. Causes irritation with burning pain, itching and redness. Prolonged or repeated skin contact may cause defatting and dermatitis. INHALATION: Causes respiratory irritation. Mercaptans may cause nausea and headache. Inhalation of high concentrations may cause central nervous system effects characterized by nausea, headache, dizziness, unconsciousness and coma. INGESTION: Harmful if swallowed. May cause gastrointestinal irritation with nausea, vomiting and diarrhea. May cause muscle paralysis, respiratory failure and possible death. Irritation, mutation and toxicity data for 2-Mercaptoethanol listed in RTECS under KL5600000.

FOR POTASSIUM ACETATE:
Oral Rat LD50 = 3250 mg/kg. EYES: Causes eye irritation. May cause chemical conjunctivitis. SKIN: Causes skin irritation.

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 - This material does not have an RQ or TPQ.
SARA 313 - This material is not reportable under 313.
EPA TSCA Section 8(b) - For Glycerol, Potassium Acetate and 2-Mercaptoethanol: Chemical Inventory.
8(d) - For Glycerol: unpublished health/safety studies.
Exposure Limits - For Glycerol: ACGIH TLV TWA: 10 mg/m3 (total particulate). OSHA PEL TWA: 15 mg/m3 (total dust).
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.