

# Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit

**Product Number 78480**

**20 reactions**

## **STORAGE**

Store at -20°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.**



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## QUALITY CONTROL

The reagents in this kit have been carefully optimized to yield a high success rate in creating single or multiple mutations in a target plasmid. USB recommends that this kit be used as directed to achieve these results. The USB Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit has been tested to yield over 75% mutated pUC19 molecules using the control mutagenic and Amp REV primers.

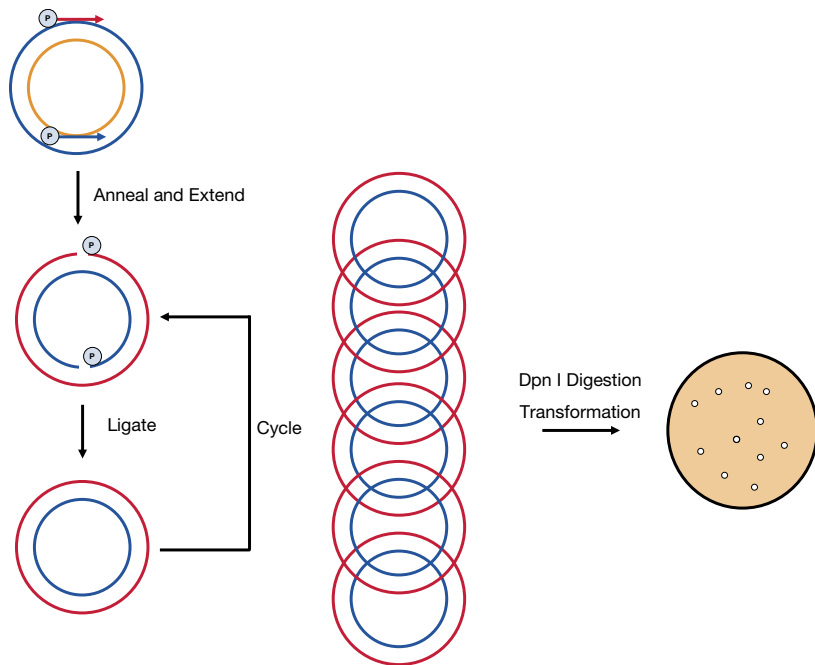
## SAFETY WARNINGS AND PRECAUTIONS

**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.

## INTRODUCTION

The Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit is designed to create single or multiple oligonucleotide-directed base changes in plasmids. The site and the specific base changes are specified by 5'-phosphorylated oligonucleotides identical to the template DNA at the 5'- and 3'-ends but with mutations (insertions, deletions, or base changes) in the middle. The template plasmid must be methylated and replication competent. The kit is based on the ligation-during-amplification procedure of Chen and Ruffner<sup>(1)</sup> however the reaction conditions have been optimized for high fidelity and efficient ligation.



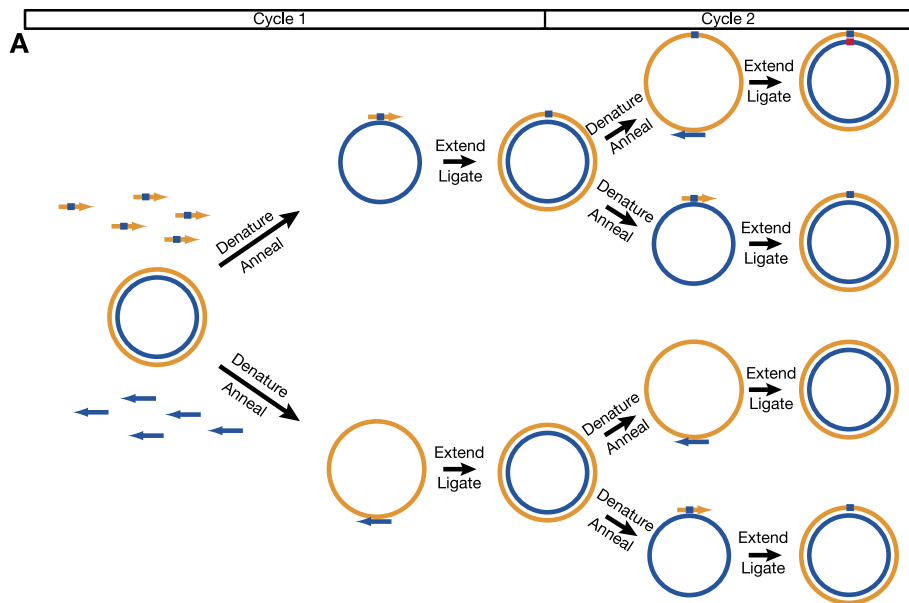
**Figure 1. Change-IT™ Multiple Mutation Site Directed Mutagenesis procedure.** During each PCR cycle the phosphorylated mutagenic primers anneal to the template plasmid and are extended by Fidelity™ DNA Polymerase. The newly synthesized DNA is ligated together by thermostable DNA ligase. Once the PCR amplification is complete, the product is digested with Dpn I and transformed into *E. coli*.

Figure 1 presents an overview of the Change-IT™ procedure. Phosphorylated primers are annealed to each strand of the plasmid template. Although only a single primer is shown per strand, each strand can have multiple primers annealed in order to create multiple mutations. Fidelity™ DNA Polymerase extends from each primer until the phosphorylated 5'-end of either the original or another primer is encountered. Thermostable ligase joins the DNA product(s) to create circular dsDNA. This dsDNA becomes the template for subsequent cycles of PCR amplification. The product of the Change-IT™ reaction is then digested with Dpn I to remove parental template and transformed into *E. coli*. Bacterial colonies bearing mutated plasmid can then be recovered.

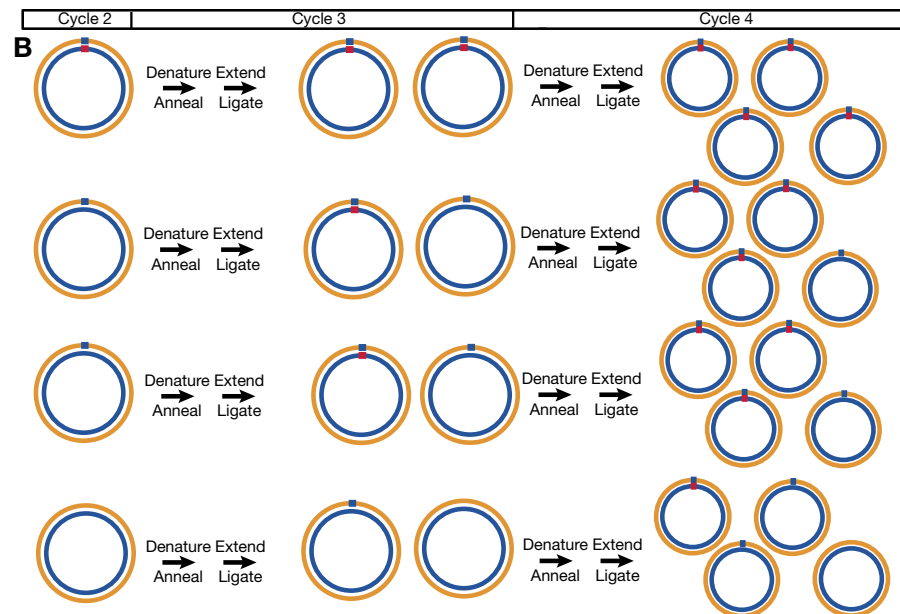
The key to the Change-IT™ technique is that the fully mutated, fully transformable plasmid is exponentially amplified while hemi-mutated plasmid is linearly amplified and non-mutated plasmid is not amplified at all. Figures 2A and 2B present an idealized version of this process for a single plasmid molecule.

Initially, template plasmid and primers are present in the reaction mixture (Fig. 2A, Cycle 1). As the first cycle progresses, the double-stranded template plasmid is melted into two single-stranded circular DNA molecules. As the temperature decreases, primer anneals to the circular ssDNA and the polymerase elongates from the 3'-end of the primer around the circle until the nascent DNA strand abuts the 5'-end of the primer, thus creating a dsDNA plasmid with the newly polymerized strand bearing a gap in the DNA. Thermal stable ligase seals the gap creating a replicated, double-stranded plasmid DNA. Note that in this round, the base change encoded by the mutagenic primer is incorporated into only one of the two DNA strands of only one of the two replicated plasmids. During the second round of PCR (Fig. 2A, Cycle 2) the two product plasmids are denatured resulting in four ssDNA closed circles, one of which bears the desired mutation. The primers anneal and extension and ligation ensue. The products from the second round of PCR amplification are one fully mutated plasmid, two hemi-mutated plasmids, and one unmutated plasmid. Figure 2B, cycle 2, recapitulates the products present at the end of cycle 2. Each of these plasmids undergo denaturation, annealing, extension, and ligation, resulting in eight plasmids (Fig 2B, Cycle 3), four of which are fully mutated, three are hemi-mutated and one is unmutated. Fig. 2B, Cycle 4, depicts the population of products present at the end of the fourth round of PCR amplification: eleven fully mutated plasmid molecules, 5 hemi-mutated molecules, and a single unmutated plasmid molecule.

After 10 cycles the ratio of mutated to unmutated plasmid is 1,000:1, and that of mutated to hemi-mutated plasmid 100:1. However, after 20 cycles these ratios are 1,000,000:1 and 50,000:1. Of course, this is an idealized representation of a complex process and assumes a PCR efficiency of 100%. Nonetheless, after 25 to 35 PCR cycles and Dpn I digestion of the parental plasmid, this technique creates a plasmid population composed of over 70 to 90% fully mutated plasmids in control reactions.



**Figure 2A. Amplification of a Single Template Plasmid in the Change-IT™ Multiple Mutation Site Directed Mutagenesis Reaction.** Figure 2A, Cycle 1, depicts the initial conditions: a single dsDNA plasmid, phosphorylated mutagenic (orange arrow with blue box) primers, and phosphorylated non-mutagenic (blue arrow) primers. In the first cycle, denaturation leads to two single-stranded DNA circles, and primers anneal as the reaction temperature decreases. FidelityTaq™ DNA Polymerase elongates from the 3'-end of the primer until the nascent DNA strand encounters the phosphorylated, 5'-end of the primer. DNA Ligase seals the gap between the 5'-phosphorylated end of the primer and the 3'-end of the newly synthesized DNA strand resulting in two plasmids, one without any mutations and one with the desired mutation in a single strand of the double-stranded plasmid. In the second cycle (Fig. 2A, Cycle 2) denaturation results in four single-stranded DNA circles, one of which bears the desired mutation. Annealing, extension and ligation result in a single plasmid with the desired mutation in both strands, two plasmids with the desired mutation in one of the two strands, and one plasmid without any mutations.



**Figure 2B. Amplification of a Single Template Plasmid in the Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit continued.** Figure 2B, Cycle 2 pictorially depicts the plasmid population at the end of the second cycle. Figure 2B, Cycle 3 depicts this population after the third cycle and Figure 2B, Cycle 4 depicts this population after the fourth cycle. Each fully mutated plasmid generates two fully mutated products; each hemi-mutated plasmid generates a single fully mutated plasmid and a single hemi-mutated plasmid; each non-mutated plasmid generates a single hemi-mutated plasmid and a single non-mutated plasmid.

## COMPONENTS

**10X Change-IT™ Buffer:** A Tris-HCl based buffer which includes 10mM MgCl<sub>2</sub>, equimolar concentrations of dATP, dGTP, dCTP, dTTP, and salts. The buffer has been optimized for high fidelity amplification and efficient ligation.

**Change-IT™ Enzyme:** A blend of Fidelity™ DNA Polymerase and a thermal stable ligase in 50% glycerol.

**Dpn I:** This restriction enzyme cuts at methylated GATC sites. It is used to remove the parental (*i.e.* non-mutated) plasmid DNA. Dpn I is supplied in 50% glycerol.

**Amp FWD Primer:** This primer is 5'-phosphorylated and is included at 5μM in Tris-HCl Buffer. The primer is complementary to the antisense strand of β-lactamase and so is oriented in the forward direction. This primer is included as a universal primer. The sequence is:  
PO<sub>4</sub>/CCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC.

**Amp REV Primer:** This primer is 5'-phosphorylated and is included at 5μM in Tris-HCl Buffer. The primer is complementary to the sense strand of β-lactamase and so is oriented in the reverse direction. This primer is included as a universal primer. The sequence is:  
PO<sub>4</sub>/GTCAGAAAGTAAGTTGCCGCAGTGTTATCACTCATGG.

**Mutagenic Control Primer:** This primer is 5'-phosphorylated and is included at 5μM in Tris-HCl Buffer. This primer binds to the antisense strand of the α-fragment of β-galactosidase and so is oriented in the forward direction. It is included as the mutagenic primer in the pUC19 control reaction. The mutation inserted by this primer prevents α-complementation and so renders bacterial colonies white on X-GAL plates. The sequence is:  
[PO<sub>4</sub>/ATGACCATGATTACGCCATAGCTTGCATGCCTGCAGG.](#)

**0.5 ng/μl pUC19:** This plasmid is provided for use as the template in the control reaction.

**Nuclease-Free Water**

## MATERIALS NOT SUPPLIED

**Necessary Reagents:**

**Plasmid template:** Plasmid DNA used in this kit should be pure and free from contaminants that might interfere with PCR amplification. We recommend that the plasmid be purified using an anion exchange resin, such as that found in the USB PrepEase® MiniSpin Plasmid Kits (PN 78735, 78736, or 78737). Plasmid concentration should be determined by absorbance at 260 nm so that

the template concentration guidelines can be followed. The plasmid template must be methylated so that it can be cleaved by Dpn I. Most *E. coli* cell lines in common use are *dam*<sup>+</sup> and will generate methylated plasmid DNA which will be digested by Dpn I. Do not use plasmid isolated from *dam*<sup>-</sup> *E. coli* cell lines, such as JM110 or its derivatives, SCS110 or INV110.

**Mutagenic primers:** Proper design of the oligonucleotide primers is critical for the success and efficiency of the Change-IT™ mutagenesis amplification. Mutagenic primers must be designed so that each binds to a unique site with no overlap between them. The bases encoding the mutation(s) should be located toward the middle of the primer and be flanked by stretches of 10 to 20 bases that are complementary to the target DNA strand. Note that the 5'-end of the primer must be phosphorylated and match the template sequence exactly for ligation to occur. It is essential for successful mutagenesis that at least one primer anneals to each DNA strand of the plasmid. To facilitate this, both Amp FWD and Amp REV primers are included.

**Highly competent cells:** Either chemically competent or electrocompetent cells are adequate, but they should have competencies of 10<sup>8</sup> cfu/μg pUC19 for 3 kb plasmids and of 10<sup>9</sup> cfu/μg pUC19 for 8 kb plasmids.

**Nutrient agar plates and growth medium:** Agar plates with the appropriate antibiotic are required for the growth of transformed *E. coli* cells and agar plates that also include X-Gal are required for the control reaction. Growth media is required during the transformation procedure.

**Necessary equipment:**

**Other supplies:** A microcentrifuge, appropriate tubes, pipette tips, and pipettors are required. The use of barrier-tip pipettes and dedicated pipettors are recommended in order to avoid contamination. PCR reaction tubes do not have to be thin walled, but should be nuclease-free. Adjust denaturing and annealing times appropriately for non-thin walled tubes.

**Latex gloves (powder free):** Gloves should be used for handling reagents and equipment in order to decrease the probability of introducing contaminants into samples.

**Thermal cycler:** A thermal cycler is required for this kit. Purchase of this kit provides a limited license for the use of the reagents in the PCR process. This license does not extend to the thermal cycler.

**Bacterial incubator:** A temperature controlled incubator for the bacterial plates is required.

## PROTOCOL

This protocol specifies appropriate volumes for a single 20 µl mutagenesis reaction. The kit itself provides sufficient material for 20 independent reactions. For multiple reactions scale the component volumes proportionately. Assemble a control reaction using 10 µl of 0.5 ng/µl pUC19, 1 µl Mutagenic Control Primer, and 1 µl Amp REV Primer.

1. Before first use, centrifuge the Change-IT™ Enzyme and Dpn I tubes to collect contents at bottom of tube.
2. Thaw 10X Change-IT™ Buffer, mix thoroughly, centrifuge to collect at the bottom of the tube, and place on ice.
3. Assemble reactions on ice. Note that at least one primer must anneal to each DNA strand of the plasmid (e.g. in a two primer reaction the common primer must anneal to the opposite strand of the plasmid as the mutagenic primer). Do not include both common primers (i.e. Amp REV and Amp FWD) in the same reaction.
4. The plasmid DNA volume added will depend on the template plasmid concentration and the number of reactions being assembled. Therefore, the volume to be added is undefined in the table below. However, the sum of the volume of plasmid DNA and the volume of water must equal 15.2 µl. The final plasmid mass in a single reaction should range from 5 ng (for a 3 kb plasmid) to 15 ng (for an 8 kb plasmid). Greater amounts of template can be used, but background colonies may increase.

Component	Volume (µl)	Final Concentration
10X Change-IT™ Buffer	2.0	1X
5µM Phosphorylated Mutagenic Primer	1.0	0.25µM
5µM Phosphorylated Common Primer	1.0	0.25µM
Methylated Plasmid DNA	X	>5 ng
Nuclease-Free Water	(15.2-X)	NA
Change-IT™ Enzyme	0.8	NA

5. Cycle reactions according to the following guidelines:

Cycle Name	Temperature	Time	Comments
Initial Denature	95°C	2 minutes	
20 to 30 cycles	Denature	95°C	30 sec
	Anneal	55°C to 68°C	30 sec
	Extend	68°C	10–20 minutes
Final Extension & Ligation	68°C	10–20 minutes	
Hold	4°C	NA	

6. Although the Change-IT™ reaction product can be analyzed on an agarose gel at this point, it is unlikely that a distinct product band will be seen. Continue with all of the steps of the Change-IT™ mutagenesis procedure, as mutated product is recovered upon completion of the protocol.
7. Remove a 10 µl aliquot of the completed reaction to a fresh tube. Add 1 µl Dpn I to 10 µl of mutagenesis reaction.
8. Incubate at 37°C for 2 hours.
9. Transform 2 to 4 µl of Dpn I digested reaction into 50 µl of competent cells.
10. Plate 10–100% of the transformed *E. coli* cells onto selective medium.
  - a. Plate 15% of the control reaction onto plates containing ampicillin and X-Gal.
  - b. A successful control reaction will yield >75% white colonies.

## SUPPLEMENTARY INFORMATION

The Change-IT™ technique is based on PCR amplification of a complete plasmid coupled with ligation of the nicks in the strands of the dsDNA circle. Because PCR plays a crucial role in this technique, all of the standard precautions for PCR apply to this kit as well. Proper primer design, template preparation, and clean work habits all greatly increase the chances for success.

## Template

Clean template greatly increases the likelihood of success in the mutagenesis reaction. This kit has been extensively tested using plasmids isolated by anion exchange resins from USB Corporation as well as from other vendors. In addition to clean plasmid template, it is important to adhere to the recommended amounts of template. High ratios of colonies bearing mutated to those bearing non-mutated plasmids are a function of successful amplification, a low amount of template, and efficient Dpn I digestion. Clearly, these considerations can lead to opposing solutions as PCR efficiency for long PCR is enhanced by increased plasmid concentration, yet this can lead to less efficient Dpn I digestion of parental plasmid. As a rule of thumb, the smaller the plasmid size (e.g. pUC19), the lower the mass of template required and the greater the Dpn I efficiency. We recommend a parental plasmid concentration of 0.2 ng/ $\mu$ l for 2.7 kb plasmids, 0.6 ng/ $\mu$ l for 8.3 kb plasmids, and higher concentrations for larger (>10 kb) plasmids. If parental plasmid background remains high, then a second Dpn I digest may be performed.

## Primer Design

Primers should be designed so that bases not matched to the template (*i.e.* mutagenic) are in the middle of the primer. At least 10 and preferably 15 to 20 bases should extend both upstream and downstream from the section of the primer which encompasses the mutagenic section. Large (300 bp) deletions have been created using this kit by designing an oligonucleotide which extends for 25 nt upstream and 25 nt downstream of the deletion boundaries. One of the greatest obstacles to success in the Change-IT™ mutagenesis reaction is failure to allow for a sufficient number of bases upstream and downstream from the mutagenic section of the primer.

The non-mutagenic primer should be designed so that it binds to a unique sequence on the opposite strand of the plasmid. This non-mutagenic primer should not overlap with the mutagenic primer in any way. This will avoid the formation of primer-dimers that are so prevalent in other mutagenesis techniques. For the users' convenience, the kit includes primers to the Amp<sup>R</sup> sequence in both the forward and reverse orientations. These primers may be used in any mutagenesis reaction where the template plasmid bears the Amp<sup>R</sup> ORF. Note that for the control reaction, the Amp REV primer should be used in conjunction with the mutagenic control primer and pUC19. If two mutations on separate primers are being introduced then the two mutagenic primers can anneal to opposite strands of the plasmid and the non-mutagenic primer may be omitted.

## Cycling Conditions

The Change-IT™ Kit uses a high fidelity polymerase blend, Fidelity™ DNA Polymerase, to amplify the plasmid. At USB we have found little difference between error rates when amplifying and ligating 20 versus 35 cycles. Consequently, we recommend that the Change-IT™ amplification be carried out for 20 to 35 cycles in order to increase the proportion of mutated plasmid molecules in the reaction. For smaller plasmids (2.8 kb) 20 cycles is sufficient; for larger plasmids (8 kb), 35 cycles may be required to achieve a greater than 75% mutation rate (defined as the number of *E. coli* colonies bearing mutated plasmid versus the number bearing parental plasmid).

The annealing temperature in these reactions is not as critical as in a typical PCR reaction. If the guidelines for primer design are followed, then an annealing temperature of 55°C works well for most primers. Note that an extension temperature of 68°C should be followed regardless of the PCR product size. The extension time in the Change-IT™ reaction is considerably lengthened as compared to a typical PCR reaction. This is to allow the thermal stable ligase sufficient time for ligation of the newly synthesized, nicked circles of DNA into closed, circular dsDNA. In general, for the extension step in the PCR cycle, allow 1 minute per kb of linear plasmid length for extension and an additional five minutes for ligation. The recommended addition of 5 minutes to the extension time is sufficient in most cases. However, for larger plasmids (>8 kb) we have noted a modest increase (~5%) in the success of the reaction using a ten, rather than five, minute addition to the extension time.

## Transformation and Growth

The efficiency of the *E. coli* cells used to transform the Change-IT™ mutagenesis reaction can vary depending on the size of the plasmid being altered. Larger (>8 kb) plasmids will require more efficient cells ( $10^8$  to  $10^9$  cfu/ $\mu$ g DNA) and that a greater percentage of the rescue, up to 100%, be plated.

## Large Plasmids

Change-IT™ mutagenesis of large (>14 Kb) plasmids may require slight modifications to the protocol. At USB we have had success by increasing the amount of template, performing two sequential Dpn I digestions, and by doubling the volume of enzyme blend used in the reaction.

## TROUBLESHOOTING

Problem	Possible Causes and Solutions
<b>High background of bacterial colonies bearing parental plasmid</b>	<ol style="list-style-type: none"> <li>1. Decrease the mass of parental plasmid used as template</li> <li>2. Increase the units of Dpn I used in the post-amplification digestion.</li> <li>3. Add an additional Dpn I digestion immediately following the first one.</li> <li>4. Increase the number of PCR amplification cycles.</li> </ol>
<b>No mutations detected</b>	Perform control mutagenesis reaction.
<b>Colonies failed to grow</b>	<ol style="list-style-type: none"> <li>1. Perform control mutagenesis reaction.</li> <li>2. Increase the number of amplification cycles.</li> <li>3. Ensure correct antibiotic used.</li> <li>4. Plate a larger volume of the <i>E. coli</i> transformation.</li> <li>5. Check the competency of the competent cells.</li> </ol>

## RELATED PRODUCTS

### PCR Master Mixes

Product	Application	Pack size	Product number
FideliTaq™ PCR Master Mix (2X)	PCR amplification	100 reactions	71182
RubyTaq™ PCR Master Mix (2X)	PCR amplification	100 reactions	71191
Taq PCR Master Mix (2X)	PCR amplification	100 reactions	71162

### Plasmid Purification

Product	Application	Pack size	Product number
PrepEase® MiniSpin Plasmid Kit	Plasmid isolation	10 preps 50 preps 250 preps	78735 78736 78737

### Ultrapure Antibiotics

Product	Application	Pack size	Product number
Ampicillin, Sodium Salt	Bacterial selection	5 gm 25 gm 100 gm	11259
Chloramphenicol	Bacterial selection	5 gm 25 gm 100 gm 1 kg	23660
Kanamycin Sulfate	Bacterial selection	5 gm 25 gm	17924
Streptomycin Sulfate	Bacterial selection	10 gm 25 gm 100 gm 1 kg	21865

### Ultrapure Antibiotics (continued)

Product	Application	Pack size	Product number
Tetracycline, Hydrochloride	Bacterial selection	25 gm 100 gm 1 kg	22105

### Reagents

Product	Application	Pack size	Product number
Agarose – Separation ≥ 500 bp, Genetic Performance Certified	DNA separation	25 gm 100 gm 250 gm 500 gm	75817
Ethidium Bromide Drops	DNA visualization	5 ml	75816
TAE Buffer, 10X Solution	DNA visualization	1 L 5 L	75904
TAE Buffer, 50X Solution	DNA visualization	100 ml	74015
TBE Buffer, 10X Ready-Mixed Powder	DNA visualization	6 x 200 ml	70454
TBE Buffer, 5X Solution	DNA visualization	1 L 5 L	75891
LB Agar, Ready-Made Powder	Bacterial growth	250 gm 1 kg	75851
LB Broth, Ready-Made Powder	Bacterial growth	250 gm 1 kg	75852
Terrific Broth, Ready-Made Powder	Bacterial growth	250 gm 1 kg	75856
X-Gal	Bacterial screening	100 mg 250 mg 1 gm 2 gm	10077
X-Gal-IPTG Solution	Bacterial screening	1.5 ml 10 ml 50 ml	26205

## REFERENCE

1. Chen, Z. and Ruffner, D. E. (1998) *Nucl. Acids Res.* 26(4), 1126–1127.

## USB CORPORATION

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

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

USB products distributed outside the USA:  
Please visit the USB website at [www.usbweb.com](http://www.usbweb.com) for up-to-date contact information within your area.

## Material Safety Data Sheet

Revision: 10/09/2006

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

**PRODUCT NAME:**  
Change-IT™ Multiple Mutation Site Directed Mutagenesis Kit

#### SUPPLIER:

**USB Corporation**  
26111 Miles Road, Cleveland, Ohio 44128 Phone: (216) 765-5000  
Please visit our website at [www.usbweb.com](http://www.usbweb.com) for contact information on USB product distributors within your area.

**PRODUCT CODE:**  
78480

**EEC NUMBER:**  
None

#### EMERGENCY CONTACT:

**Chemtrec: (800) 424-9300**  
**Outside USA & Canada: 703-527-3887**

### COMPOSITION/

#### HAZARDOUS COMPONENTS

#### HAZARD

For components 78482  
& 78483:  
Glycerol

#### CAS NO.

56-81-5

#### %WT

~50%

#### TLV

ACGIH TLV - TWA:  
10 mg/m<sup>3</sup> (total particulate)  
OSHA TWA: 15 mg/m<sup>3</sup>  
(total dust)

#### CHIP R & S PHRASES

R:36/37/38 Irritating to eyes, respiratory system and skin.  
S:26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S:36/37 Wear suitable protective clothing and gloves.

#### For component 78481:

Potassium Chloride 7447-40-7 -3.7% -

Tris-HCl

1185-53-1 -3.9% -

R:36/37/38 Irritating to eyes, respiratory system and skin.

S:23 Do not breathe vapour.

S:24/25 Avoid contact with skin and eyes.

S:36/37 Wear suitable protective clothing and gloves.

### HAZARDS IDENTIFICATION

**CHIP**

Irritant

**HCS**

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 Glycerol: Contact with strong oxidizing agents may produce an explosion.

Explosion Limits for Glycerol = Lower - 1.1; Upper - Not available.

Flash point for Glycerol = 193°C (379.4°F); Autoignition temperature for Glycerol = 400°C (752°F).

### ACCIDENTAL RELEASE MEASURES

Wear appropriate personal protective equipment and clothing including lab coat, safety glasses, gloves and NIOSH-approved respirator. Ventilate the area and stop the leak if it can be done without risk, dilute with water before mopping or take up with sand, earth, or other absorbing material. Place material in a suitable dry, leak-proof 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. Wash thoroughly after handling. Use with adequate ventilation. Wash clothing before reuse. Store at -20°C. Containers (even empty) may retain product vapors and residue. Store away from ignition sources and excess heat. Store away from incompatible materials including strong oxidizers, strong acids, mixtures with hydrogen peroxide, potassium permanganate, trifluorobromide, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide.

### 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: Vials of solutions  
 Vapor Pressure: No data available  
 Solubility (Water): Soluble  
 Percent Volatile: No data available  
 Chemical Formula: Kit

Boiling Point: No data available  
 Vapor Density: No data available  
 Specific Gravity: No data available  
 Evaporation Rate: No data available

## **STABILITY AND REACTIVITY**

Product is stable under normal conditions. Avoid strong oxidizing agents including mixtures with hydrogen peroxide, potassium permanganate, trifluorobromide, calcium hypochlorite, nitric acid, sulfuric acid, perchloric acid and lead oxide. For Glycerol: Contact with Sodium Hypochlorite and Hypochlorous acid may cause an explosion.

## **TOXICOLOGICAL INFORMATION**

### **EFFECTS OF OVEREXPOSURE:**

**EYES:** Contact may cause irritation and slight corneal injury.

**SKIN:** Prolonged contact may cause irritation and/or allergic reaction.

**INHALATION:** May cause irritation to mucous membranes and upper respiratory tract.

**INGESTION:** Chronic ingestion or excessive dosage may cause irritation of the gastrointestinal tract with nausea, vomiting and diarrhea.

### **ADDITIONAL INFORMATION:**

Glycerol: Irritation, mutation, reproductive effects and toxicity data listed in RTECS under MA8050000.

Irritation data: Skin Rabbit = 500 mg/24H Mild (1986). Eye Rabbit = 500 mg/24H Mild (1986). Toxicity data: Oral Rat LD50 = 12600 mg/kg (1945). Inhalation Rat LC50 = >570 mg/m3/1H (1970).

Potassium Chloride: Irritation, mutation and toxicity data listed in RTECS under TS8050000. Irritation data: Eye Rabbit = 500 mg/24H Mild (1972).

Toxicity data: Oral Rat LD50 = 2600 mg/kg (1972). Details of toxic effects not reported other than lethal dose value.

Laboratory experiments have resulted in mutagenic effects.

Tris-HCl: RTECS: No data available.

Only select RTECS information is provided here. Please see actual RTECS entries for both Glycerol and Potassium Chloride for complete information.

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

No information available.

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

US DOT / IATA: No applicable information.

## **ECOLOGICAL INFORMATION** **DISPOSAL CONSIDERATIONS** **TRANSPORTATION 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, Tris-HCl and Potassium Chloride: Chemical Inventory.

Exposure Limits Glycerol – ACGIH TLV TWA: 10 mg/m3 (total particulate).

OSHA PEL TWA: 15 mg/m3 (total dust).

California Proposition 65 – No applicable information.

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**USB Corporation**  
26111 Miles Road  
Cleveland, Ohio 44128 USA  
**www.usbweb.com**

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