Alkali-Cation Yeast Transformation Kit

For the high efficiency transformation of library DNA into yeast two-hybrid reporter strains

Revision # 2200-999-1J10

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Application Manual

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Product Description	# of Preps	Cat #.
Alkali-Cation Yeast Transformation Kit	250	2200-200

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1. Introduction

1.1 Alkali-Cation Yeast Transformation Kit Introduction

The Alkali-Cation Yeast Transformation Kit provides all of the reagents required to perform quick and easy non-electrical transforming of yeast with linear or plasmid DNA. Host cells are made competent by treatment with a Lithium/Cesium Acetate mixture. The entire procedure may be completed in less than 90 minutes and routinely provide a transformation efficiency of greater than 10^4 transformants per µg of DNA using YEP24 plasmid DNA.

1.2 Related Products

Yeast Kits and Accessories

Yeast SpheroplastTransformation Kit

Application: Screening cDNA libraries for complementation, or for construction of YAC libraries

Quantity	Cat #
25 preps	2210-200

EZ Yeast Transformation Kit

Application: High throughput transformation without making competent cells

Quantity	Cat #
200 preps	2100-200

Whole Cell Yeast PCR Kit

Application: High throughput direct PCR amplification of yeast colonies without purifying DNA

Quantity	Cat #
500 preps	2016-200

Yeast Cell Lysis Kit

Application: Used with GNOMEÆ DNA Isolation Kit to isolate yeast genomic DNA

Quantity	Cat #
100 preps	2015-600

Yeast RPM® Kit

Application: Isolation of plasmid DNA from yeast

Quantity	Cat #
100 preps	2069-400

FastDNA® Kit

Application: Isolation of genomic DNA from yeast or virtually any source

Quantity	Cat #
100 preps	6540-400

FastRNA® Kit, Red

Application: Isolation of total RNA from yeast, algae or fungi

Quantity	Cat #
100 preps	6030-600

FastPROTEIN™ Red Kit

Application: Isolation of protein from yeast

Quantity	Cat #
50 preps	6550-600
100 preps	6550-700

Library-in-a-Tube

Application: A PCR-ready single-stranded cDNA library made from total RNA for single-use PCR reactions.

Description	Size	Cat #
Yeast: *S. cerevisiae*, Stationary Phase	3 x 0.2 ml	5610-134-1
Yeast: *S. cerevisiae*, Stationary Phase	3 x 0.5 ml	5610-134-2
Yeast: *S. cerevisiae*, Log Phase	3 x 0.2 ml	5610-135-1
Yeast: *S. cerevisiae*, Log Phase	3 x 0.5 ml	5610-135-2
Yeast: *S. pombe*, Stationary Phase	3 x 0.2 ml	5610-138-1
Yeast: *S. pombe*, Stationary Phase	3 x 0.5 ml	5610-138-2
Yeast: *S. pombe*, Log Phase	3 x 0.2 ml	5610-139-1
Yeast: *S. pombe*, Log Phase	3 x 0.5 ml	5610-139-2
Yeast: *S. albicans*, Stationary Phase 90235	3 x 0.2 ml	5610-150-1
Yeast: *S. pombe*, Stationary Phase 90235	3 x 0.5 ml	5610-150-2
Yeast: *S. pombe*, Log Phase 90235	3 x 0.2 ml	5610-151-1
Yeast: *S. pombe*, Log Phase 90235	3 x 0.5 ml	5610-151-2
Yeast: *S. albicans*, Stationary Phase 90236	3 x 0.2 ml	5610-152-1
Yeast: *S. pombe*, Stationary Phase 90236	3 x 0.5 ml	5610-152-2
Yeast: *S. pombe*, Log Phase 90236	3 x 0.2 ml	5610-153-1
Yeast: *S. pombe*, Log Phase 90236	3 x 0.5 ml	5610-153-2

Replica Plating Apparatus

This hardwood replica plating apparatus is made from fallen trees. It can be used with velvet, gauze, or even paper towels.

Description	Quantity	Cat #
Replica Plating Apparatus (100 mm)	1	5000-001
Replica Plating Apparatus (150 mm)	1	5000-004
Velvet Pad for 100 mm plates (6 sq in.)	1	5000-006
Velvet Pad for 100 mm plates (6 sq in.)	10	5000-007
Velvet Pad for 150 mm plates (9 sq in.)	1	5000-008
Velvet Pad for 150 mm plates (9 sq in.)	10	5000-009

Yeast Growth Media

Standard Formulations

YPD (YEPD) Broth

Contents/L: 20 g peptone, 10 g yeast extract-Y, 20 g dextrose

Description	Quantity	Cat #
Large Capsules	227 g	4001-016
Capsules	227 g	4001-011
Powder	227 g	4001-012
Pouch, 0.5 L	10 x 0.5 L	4001-065
Pouch, 1.0 L	10 x 1.0 L	4001-075

YPD Agar

Contents/L: YPD, 17 g Agar-Y

Description	Quantity	Cat #
Capsules	227 g	4001-211
Powder	227 g	4001-212
Pouch, 0.5 L	10 x 0.5 L	4001-265
Pouch, 1.0 L	10 x 1.0 L	4001-275

DOB

Contents/L: 1.7 g YNB, 5 g Ammonium Sulfate, 20 g Dextrose

Description	Quantity	Cat #
Powder	227 g	4025-012
Pouch, 0.5 L	10 x 0.5 L	4025-065
Pouch, 1.0 L	10 x 1.0 L	4025-075

DOBA

Contents/L: DOB, 17 g Agar-Y

Description	Quantity	Cat #
Powder	227 g	4025-212
Pouch, 0.5 L	10 x 0.5 L	4025-265
Pouch, 1.0 L	10 x 1.0 L	4025-275

YNB

Contents/L: Standard Formulation

Description	Quantity	Cat #
Powder	227 g	4027-012

YNB w/ Ammonium Sulfate

Contents/L: YNB, 5 g Ammonium Sulfate

Description	Quantity	Cat #
Powder	227 g	4027-512

Complete Supplement Mixture (CSM)

Description	Quantity	Cat #
CSM powder	10 g	4500-012
CSM-HIS powder	10 g	4510-312
CSM-LEU powder	10 g	4510-512
CSM-TRP powder	10 g	4511-012
CSM-URA powder	10 g	4511-212

** Other formulations available*

Pre-poured Plates

Description	Quantity	Cat #
YPD Agar	10 plates	4001-224
CSM Complete	10 plates	4800-124
CSM-HIS	10 plates	4810-124
CSM-LEU	10 plates	4811-124
CSM-TRP	10 plates	4812-124
CSM-URA	10 plates	4813-124

**Other formulations available*

Yeast Growth Media Additives and Components

Agar-Y

Description	Quantity	Cat #
Capsules	227 g	4019-011
Powder	227 g	4019-012

5-FOA

Description	Quantity	Cat #
Powder	1 g	4066-102

3-AT

Description	Quantity	Cat #
Powder	50 g	4061-722

1.3 Key Features and Applications

- Easy and quick non-electrical method for transforming yeast with linear or plasmid DNA
- The entire procedure may be completed in less than 90 minutes
- Provides a transformation efficiency of greater than 10^4 transformants per µg of DNA using YEP24 plasmid DNA

2. Alkali-Cation Yeast Transformation Kit Components

Catalog # 2200-200 (250 preps)

Description	Volume	Cat #
TE, pH 7.5, sterile solution	275 ml	2200-201
Lithium/Cesium Acetate	125 ml	2200-202
Carrier DNA, 10 mg/ml, sterile	1.25 ml	2200-205
Histamine, sterile solution	1.25 ml	2200-207
PEG	200 ml	2200-203
TE/Cation MIXX	50 ml	2200-204
SOS, sterile solution	40 ml	2200-206

3. Storage of Alkali-Cation Yeast Transformation Kit

The Alkali-Cation Yeast Transformation Kit is shipped at ambient temperature. Upon receipt, store at 4°C.

4. Alkali-Cation Yeast Transformation Kit Protocol

4.1 Things to Know Before Using the Alkali-Cation Yeast Transformation Kit

If top agar is used for plating transformed yeast, it should be identical to the plate agar except with half the concentration of agar. It should not be warmer than 50°C.

4.2 Alkali-Cation Yeast Transformation Kit Protocol

1. Inoculate a single colony into 100 ml YPD (YEPD) Broth (BIO 101® Systems catalog # 4001-021) and grow with aeration at 30°C to mid log, 2 x 10^6 to 2 x 10^7 cells/ml. (Approximately 12-24 hours).
2. Spin to pellet cells at 400 x g for 5 minutes; discard supernatant.
3. Resuspend cells in a total of 9 ml TE, pH 7.5. Spin to pellet cells and discard supernatant.
4. Gently resuspend cells in 5 ml Lithium/Cesium Acetate Solution.
5. Incubate at 30°C for 30 minutes with gentle shaking.
6. Spin at 400 x g for 5 minutes to pellet cells and discard supernatant.
7. Gently resuspend in 1 ml TE, pH 7.5. Cells are now ready for transformation.
8. In a 1.5 ml tube combine:
   - 100 µl yeast cells
   - 5 µl Carrier DNA
   - 5 µl Histamine Solution
   - 0.01-1 µg plasmid DNA in a 10 µl volume (max)

Gently mix and incubate at room temperature for 15 minutes.

9. In a separate tube, combine 0.8 ml PEG and 0.2 ml TE/Cation MIXX for each transformation reaction. Add 1 ml of this PEG/TE/Cation MIXX to each transformation reaction. Mix cells into solution with gentle pipetting.
10. Incubate at 30°C for 10 minutes.
11. Heat shock at 42°C for 10 minutes; cool to 30°C.
12. Pellet cells in a microcentrifuge on high power for 5 seconds and remove supernatant.
13. Resuspend in 200 µl SOS and plate in appropriate selective media either directly or in top agar. (see "Things to know before using the Alkali-Cation Yeast Transformation Kit"). Incubate at 30°C for 48-72 hours until transformant colonies appear.

5. Appendix

(i) Product Use Limitation & Warranty

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