SCOS

Saccharomyces cerevisiae One-Step Transformation Kit

Protocol ver. 951004

Notes on Shipping & Storage:

Shippment: The components of **SCOS** kit should be shipped at 2~8 ºC
Storage: see page 3.

Yeastern Biotech Co., Ltd.
http://www.yeastern.com
E-mail : yeastern@yeastern.com
TEL : 886-2-26954245 Fax : 886-2-26953979

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Product description

The SCOS (*Saccharomyces cerevisiae* one-step) yeast transformation kit provides an fast **one-step/one-tube** method for transforming the yeast, ***Saccharomyces cerevisiae*** with linear or circular plasmid DNA. The entire procedure may be completed in **10~60 min**. and routinely provides a transformation efficiency of **10^3~10^6** transformants per μg of plasmid DNA (variarions depend on the transformation procedure and the properties of yeast cells and plasmids).

- **Applications:** High throughput transformation without making competent cells.
- One-step and one-tube 10~60 minutes protocol for transforming the yeast, *Saccharomyces cerevisiae*: **Mix→Heat shock→Plating**
- 120 transformations for each SCOS kit
- Suitable for yeast cells from colonies, broth or any growth phase
- No more preparing yeast competent cells
- Repeatable efficiency, always reach efficiency of 10^3 ~ 10^6/μg DNA

Kit Components

Name	Volume	Shipping Temp	Storage Temp	Catalog #	Volume for each Reaction	Description
SCOS Buffer	12 ml (1.5mlx8)	2-8 °C	Room Temp	YY014-1	100 μl	A mixture of Cations and Polyethylene glycol
Carrier DNA	0.6 ml	2-8 °C	Dispense into 50 μl aliquots, stored at -20 °C	YY014-2	5 μl	A mixture of single strand DNA for enhancing the transformation efficiency
DTT powder	0.2g	2-8 °C	Add 0.65 ml dd-water and sterilized with filter, dispense into 50 μl aliquots, stored at -20 °C	YY014-3	5 μl	A reducing agent solution for enhancing the transformation efficiency

Schematic Protocol

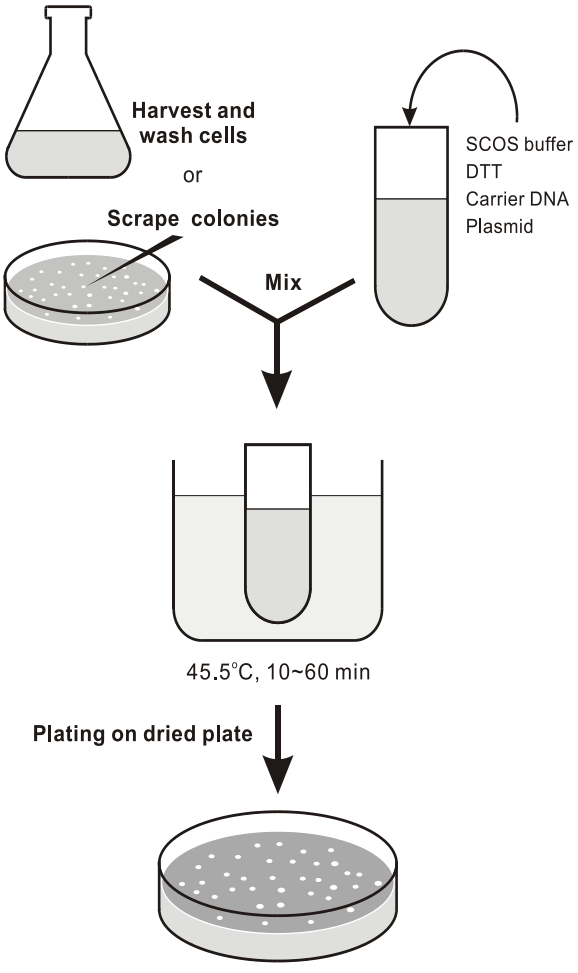
1. Preparation of cells for transformations:

i) For cells from lawn of colonies: pick up a single colony and streak or spread completely on a YPD plate and incubate at 28-32 ºC for 8- 96 hours, scrape the lawn of cells from agar surface. Mix and vortex cells with ~110 µl SCOS cocktail (fresh made, with or without plasmid)in a cell density of ~5 x10^7 cells/tube .

ii) For cells from YPD broth: pick up a fresh single colony and inoculate it into 1~10 ml YPD broth, shakes at 28~32 ºC to be saturated (20~30 hours), transfer total saturated culture into fresh 10~100 ml YPD broth for 8-24 hours (log ~stationary phase). Spin to pellet cells at 2000 x g for 10 minutes, discard supernatant. Wash twice with sterilized dd. H_2O to deplete all residues of YPD broth, resuspend and vortex cells with 110-115 µl SCOS cocktail (fresh made, with or without plasmid) in a cell density of ~5 x 10^7 cells/tube .

2. Preparation of the SCOS cocktail for each transformation:

i) Add 100 µl SCOS buffer in 1.5ml tube.
ii) Add 5 µl DTT (stock at -20 ºC, thaw on ice or quick thaw in room temp. water bath).
iii) Add 5 µl carrier DNA (stock at -20 ºC, thaw on ice).
iv) Add plasmid DNA (volume less than 5µl).



3. Preparation of dried selective plates:

Fully dried plates always give good transformation efficiency in Yeastern's SCOS transformation system. We suggest the plating procedure as: uncover each plate in a laminar flow for about 1 hour and certain the surface is dried before cover each plate.

4. *SCOS* Transformation:

i) Mix and vortex cells with SCOS cocktail for suspending yeast cells in SCOS transformation cocktail.
ii) Cap and incubate the tubes at **45.5 ºC** for 10~60minutes (depend on the method of cell preparation).
iii) Spread the "transformation cocktail" onto a "**dried**" selective agar (i.g. YNBD or SD agar + supplements) directly. Finish this procedure in 20 seconds. Incubate the plates at 28-32 ºC for 2-4 days.

Q& A

Q: Is the wetness of plate and the plating method affects the SCOS transformation efficiency?

A: **Fully dried plates always give good transformation efficiency in Yeastern's SCOS transformation system. Table 1 show the effect of agar wetness and solidity on the transformation efficiency. We suggest the plating procedure as :**

1. **Before plating, uncover each plate in a laminar flow for about 1 hour and certain the surface is dried before cover each plate.**
2. **While plating: we suggest using glass beads (4mm, dried) to spread the transformation cocktail, finish this procedure in 20 seconds.**
3. **After plating: seal the plates with parafilm or place them into a plastic box to prevent overdrying in a subsequent cultivation, grow transformed colonies at 28-32oC for 2-3 days.**

Table 1. Effect of agar solidity and wetness on the SCOS transformation system

Agar solidity	Agar surface wetness	Transformation efficiency
3.0%	dry	1.6×10^5
2.5%	dry	3.0×10^5
2.0%	dry	3.2×10^5
2.0%	wet	1.35×10^5
1.5%	dry	1.04×10^5

Q: How to get optimal Transformation efficiency?

A: To get highest transformation efficiency, there are some tests you can do: (refer to **Table2**)

1. **The heat shock length. Test various times from 10 min to 60 min heat shock at 45.5ºC.**
2. **The age of yeast cells. SCOS transformation kit suitable for *Saccharomyces cerevisiae* cells harvest from >24 hours colonies or 8-24 hour broth. However, for some special strains, do a time course experiment to harvest cells from different growth stage.**

Table 2. Effects of heat shock length and cell age on the efficiency of SCOS transformation system*

Yeast cells harvest from	Heat shock length	Cell ages		
		8 hours (log phase)	24 hours (early stationary phase)	60 hours (late stationary phase)
Lawn of colonies on YPD agar	10	1.5×10^4	1.5×10^4	2.3×10^3
	30	2.0×10^4	4.0×10^4	4.6×10^4
	60	5.3×10^3	4.0×10^4	2.4×10^4
YPD broth	10	2.0×10^5	7.0×10^3	
	30	2.5×10^5	3.0×10^5	
	60	9.0×10^2	1.8×10^5	

*0.1μg of a 6.8 kb control plasmid pYB-L (A 2μ-ori derived shuttle plasmid contains Leu2 marker for auxotrophic selection in leu2$^-$ strain) was used to perform all transformation with the host strain TL154 (ATCC 96030, A *trp1$^-$, leu2$^-$* strain, genotype is : *alpha, leu2, trp1, gal2, G418^s*)

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General Information

Yeastern Biotech Co., Ltd., a biotechnology company, founded in May, 2000 and has had a fully functional research facility since November of 2000. The focus of Yeastern is to work for other biotechnology companies to help them solve specific product production problems on yeast genetic engineering. The production microorganism used is mainly the over-secretion and low-glycosylation mutants of the yeast, *Saccharomyces cerevisiae*. From the beginning, Yeastern has also focused on developing and commercializing advanced techniques or reagents for the gene synthesis and competent cells preparation. We are proud to contribute to the efforts of researchers working at universities, in government laboratories, and at biotechnology and pharmaceutical companies around the world. For more information on our products and other services, please visit our web site at **www.yeastern.com** or contact us directly.