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Manual

Pichia Transformer Kit

Kit for preparation of competent *Pichia pastoris* cells and transformation. Chemical method.

cat#	size
4000-120	6 x 20 transformations

For research use only.

Guarantee

A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of contents

Contents

	4000-120	storage
S1P solution	70 ml	+4 °C
S2P solution	7 ml	+4 °C
S3P solution	7 ml	+4 °C

This kit was tested on *Pichia pastoris* GS115 strain.

pH of S1, S2, S3 solutions must be 8.3 ∓ 0.1. Different pH reduces the efficiency of the transformation. If it is too low, add NaOH, if too high - HCl.

Additional equipment and reagents

Necessary for competent cells preparation

- *Pichia pastoris* strain
- sterile YPD Broth medium (YPD) (cat.# 2027-250, 2027-500, 2027-1000)
- sterile YPD Agar medium (YPDA) (cat.# 2028-250, 2028-1000)
- sterile 1.5 ml Eppendorf tubes, sterile 50 ml Falcon tubes
- shaking incubator set to 30 °C
- centrifuge with rotor for 50 ml tubes

Necessary for competent cells transformation

- sterile YPD Broth medium (YPD) (cat.# 2027-250, 2027-500, 2027-1000)
- selection medium plates: 1 plate for 1 transformation
- sterile 50 ml Falcon tubes
- thermoblock set to 30 °C, 42 °C
- centrifuge with rotor for 50 ml tubes

Media preparation

Preparation of 1000 ml of medium.

YPD Broth (YPD) (cat.# 2027-250, 2027-500, 2027-1000)

1. Add **50 g** of medium to the appropriate vessel.
2. Add sterile water up to **1000 ml** and mix.
3. Autoclave for **10-20 min** at **121 °C**.
4. After cooling to **50-60 °C** mix again before use.

   Note: At 25 °C pH should be 7.0.

YPD Agar (YPDA) (cat.# 2028-250, 2028-1000)
YPD Agar (YPDA) with zeocin (100 µg/ml of zeocin)

1. Add **70 g** of medium to the appropriate vessel.
2. Add sterile water up to **1000 ml** and mix.
3. Autoclave for **10-20 min** at **121 °C**.
4. After cooling to **50-60 °C** add **100 ml** of **sterile 20% agarose** solution and additionally **1 ml** of **zeocin** (100 µg/ml) to the medium with zeocin. Mix again before use.

   Note: At 25 °C pH should be 7.0.

Competent cells preparation protocol

- *P.pastoris* should be streaked reductively onto YPDA medium
- Incubate plates for 2 days at 28-30 °C
- S1P and S2P solutions should be at room temp.

1. Inoculate a single colony of *P.pastoris* obtained from reduction culture into **10 ml** of **YPD medium**. Incubate **overnigh**t at **30 °C**.

2. Add **enough overnight culture** to **10 ml** of fresh **YPD medium** to obtain OD_{600}=0.1-0.2.

3. Incubate in a shaking incubator for **4-6 hours** at **30 °C** until OD_{600}=0.6-1.0.

   If after 6 hours of incubation the culture does not reach OD_{600}=0.6, strain should be streaked reductively once more and the procedure started from the beginning.

4. Centrifuge for **5 min** at **500 x g** at **room temp.**

5. Discard the supernatant.

6. Carefully resuspend the pellet in **10 ml** of **S1P solution.**

7. Centrifuge for **5 min** at **500 x g** at **room temp.**

8. Discard the supernatant.

9. Carefully add **1 ml** of **S2P** solution and gently mix.

10. Transfer **50 µl** of **competent cell suspension** to 1.5 ml tubes.

    50 µl of competent cells should be used for one transformation. Repeated freeze-thaw cycles do not affect the efficiency of the transformation.

11. Competent cells are ready for transformation (page 5.) or can be stored at -80 °C for later use.

    It is very important that competent cells are slowly frozen. Cells must not be frozen in liquid nitrogen.

Competent cells transformation protocol

- Prepare plates with a selective medium.
- Before transformation, the medium and S3P solution should be at room temp.
- It is recommended to prepare an additional plate with a selective medium for the negative control.

1. Use **50 µl** of ***P.pastoris*** **competent cells** for each transformation. Cells should be thawed on ice or freshly prepared at room temp.
2. Add **3 µg** of **linear DNA** and gently mix.

   Increasing the amount of DNA to 5 µg may increase the efficiency of the transformation.
   The volume of DNA should not exceed 5 µg.
3. Add **1 ml** of **S3P** solution and mix by vortexing.
4. Incubate for **1 hour** at **30 °C**. Vortex every **15 min**.

   Vortex every 15 min affects the improvement of transformation efficiency.
5. After incubation place the tubes in thermoblok for 10 **min** at **42 °C**.

   If transformed plasmid is without zeocin resistance follow point 7.
6. If transformed plasmid is zeocin resistant:
   - transfer the mixture to **5 ml** of **YPD media without antibiotics** in a 50 ml tube.
   - incubate for **1 hour** with shaking at **220 RPM** at **30 °C** to express genes responsible for antibiotic resistance.
7. Centrifuge for **5 min** at **3000 x g** at **room temp.**
8. Discard the supernatant.
9. Add **150 µl** of **YPD** media.
10. Cultivate **transformation mixture** on a plate with a selective medium.

    YPDA with zeocin (100 µg/ml) is recommended for zeocin resistance vectors.
11. Incubate for **2-4 days** at **30 °C**.

Using this kit allows us to obtain about 50 colonies per transformation.

Safety information

WARNING

S2P solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.
P270 Do not eat, drink or smoke when using the product.
P280 Wear protective gloves / eye protection / face protection.
P301+P312 In case of ingestion if you feel unwell, contact a Poison Center or a doctor.
P302+P352 In case of contact with the skin, wash with plenty of water.
P305+P351+P338 Ii in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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