

**P09101**

**P09102**

**P09103**

## Lytic Agent

**Product** The Lytic Agent is an enzymatic mix that has been formulated to support plasmid extraction from yeast and is used together with a standard plasmid preparation kit for *E. coli*.

**Contents** The Lytic Agent is available in three different sizes. Each package contains the Lytic Agent in lyophilized form and the appropriate amount of Lytic Agent Buffer.

Product Number	Size	Final volume of Lytic Agent Solution
P09101	96 preps	4 ml
P09102	384 preps	16 ml
P09103	3840 preps	164 ml

### Handling and storage

The Lytic Agent is supplied as a lyophilized powder, store at -20°C for up to six months. The Lytic Agent Buffer can be stored at 4°C for up to six months. Prepare the Lytic Agent Solution immediately before use: add the Lytic Agent Buffer into the vial containing the Lytic Agent and mix thoroughly by shaking. Use the Lytic Agent Solution immediately.

## Application

Plasmid extraction from yeast can be challenging, due to the stable cell wall which must be disrupted prior to plasmid extraction. There are several methods available for isolating plasmids from yeast, the most common one being mechanical disruption of yeast with glass beads, followed by phenol/chloroform extraction and isopropanol precipitation of DNA. The more convenient protocol described below combines enzymatic cell wall disruption by the Lytic Agent with a standard plasmid miniprep kit procedure. Plasmid DNA purified with this protocol can be used for downstream applications like PCR amplification or transformation of *E.coli*.

## Protocol for the extraction of plasmids from cultured yeast

1. Fill sufficient wells of a 96 deep-well master block with 1 ml appropriate liquid yeast medium.
2. Inoculate each well with approximately ¼ loop of plasmid bearing yeast cells, cover the block with breathable plate sealer and grow over night at 30°C to and OD<sub>546</sub> of 0.6 to 1, with shaking at 220 rpm.
3. Centrifuge 5 minutes at 4000x g and discard the supernatant.
4. Resuspend yeast pellets in the appropriate volume of the resuspension buffer supplied in the miniprep kit of your choice. Vortex for 1 minute.
5. Add 40 µl of the Lytic Agent Solution and mix by vortexing.
6. Incubate 30 minutes at 30°C, with shaking at 250 to 300 rpm.
7. Add the appropriate volume of the lysis solution supplied in the miniprep kit of your choice, vortex for 30 seconds and allow the sample to sit for 5 minutes at room temperature for lysis.

*Do not allow samples to lyse for longer than 10 minutes.*

*If you notice a white precipitate in the lysis solution prior to addition, warm the bottle in a 37°C water bath or under hot running water, shaking periodically, until the precipitate dissolves. It is recommended that you wear gloves when handling the lysis solution, as it is a basic solution.*

8. Add the appropriate volume of the neutralization solution supplied in the miniprep kit of your choice, mix by inversion and allow the samples to neutralize for 10 minutes.

*It is recommended that you wear gloves when handling the neutralization solution. Addition of the neutralization solution neutralizes the mixture and precipitates proteins and cellular debris, creating a white flocculent.*

9. Follow the instructions described in the manual of your miniprep kit of choice.
10. The resulting yeast DNA is a mix of plasmid and sheared genomic DNA. The DNA is not suitable for direct sequencing, but may be used as a template for PCR or for transformation into *E. coli*.  
*Use 0.5-1 µl for PCR or 5 µl for E. coli transformation.*

### Compatible miniprep kits

The following plasmid extraction kits (miniprep kits) are compatible with Lytic Agent:

- GeneJET Plasmid Kit (Fermentas)
- PureYield Plasmid System (Promega)
- NucleoSpin Plasmid Kit (Macherey-Nagel)
- QIAprep Spin Miniprep Kit (Qiagen)