

Product Information

Cosmid Construction Kit-1

Research use only

Catalog No: R-1001

Lot No: 1016

Contents:

- Lambda packaging extract, 50 µL (#R-1005)
- SM medium, 1 mL (# R-1004)
- OD101 glycerol stock, 200 µL (#R-1006)
- Maltose/MgSO₄ mix, 0.7 mL (# R-1023)
- Amp/Kan antibiotic mix, 500 µL (#R-1007)

Storage: -70 °C

Features and Applications: These reagents are used to construct at least 10 AdenoQuick cosmids.

- ◆ The lambda packaging extract is used to package the cosmid ligation products into phage λ.
- ◆ The SM medium is used to dilute the packaging reaction at the end of the 30-minutes incubation period.
- ◆ OD101 is the *E coli* strain that will be infected with the packaged phage λ.
- ◆ Maltose and MgSO₄ are added to the *E. coli* OD101 culture to induce expression of the receptor for phage λ.
- ◆ Amp/Kan antibiotic mix is added to the *E. coli* OD101 culture (Petri dish or liquid LB) to select for λ.

Note: All reagents are sterile and must be handled accordingly.

Quick protocol

Preparation of λ-competent E. coli

- Add 2 mL LB + 60 µL MgSO₄/maltose solution in a sterile 10-mL tube.
- Inoculate with one colony of an overnight culture of OD101.
- Grow at 37 °C until the culture appears cloudy.
- Chill on ice.
- Dilute the cells with LB to Abs_{600nm} = 1

Ligation

- Digest your shuttle plasmid with AlwNI, BstAPI, DraIII, PflMI, or SfiI (whichever is not present in your transgene), and purify the DNA fragment of interest on agarose gel
- Ligate 2 µL SfiI-digested AdenoQuick1.0 plasmid with 400 ng insert, for 1 hr at room temperature.

Packaging

- To a microcentrifuge tube, add:

- 2 µL ligation mix
- 5 µL Lambda packaging extract
- Incubate at 30°C for 30 minutes.
- Add 100 µL SM medium.
- Add 5 µL chloroform.
- Mix the tube until a white precipitate appears.
- Spin at 13,000 rpm for 20 sec.
- Transfer the supernatant containing the packaged phage λ to a clean tube.

Infection and clone selection

- Mix 60 µL packaged phage λ and 60 µL diluted competent cells.
- Incubate for 30 min at 37 °C.
- Add 20 µL Amp/Kan antibiotic mix and spread on a Petri dish containing ~ 20 mL LB/agar.
- Incubate overnight at 37°C.

Consult the AdenoQuick manual for details.

OD260 Inc.
info@od260.com

1-855-260-2606
www.od260.com