O.D.260 Inc.

Product Information

Cosmid Construction Kit-2

Research use only

Catalog No: R-1002
Lot No: 1016

Contents:
- Maltose/$MgSO_4$ mix, 0.7 mL (# R-1023)
- SM medium, 1 mL (# R-1004)
- Lambda packaging extract, 50 µL (#R-1005)
- OD101 glycerol stock, 200 µL (#R-1006)
- Amp/Kan antibiotic mix, 500 µL (#R-1007)
- SfiI enzyme, 70 µL (#R-1008)
- 10x SfiI buffer, 200 µL (#R-1009)

Storage: -20°C for the SfiI enzyme and 10x SfiI buffer; -70 ºC for all other reagents. .

Features and Applications: These reagents are used to construct at least 10 AdenoQuick2.0 cosmids.

♦ SfiI is used to digest the 4 adenovirus shuttle plasmids.

♦ Maltose and $MgSO_4$ are added to the *E. coli* OD101 culture to induce the expression of the receptor for phage λ.

♦ 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.

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 Abs600nm = 1

SfiI Digestion

- Digest your 4 shuttle plasmids (0.5 µg each) with SfiI in a single vial for 30 min at 50°C.

Ligation

- Mix 8 µL SfiI-digested plasmid mix + 1 µL T4 ligase + 1 µl 10x T4 ligase buffer.
- Incubate for 1 hr at room temperature for 1 hr.

Packaging
- To a microcentrifuge tube, add:
  - 2 µL ligation mix
  - 5 µL Lambda packaging extract
- Incubate at 30ºC 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.

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