O.D.260 Inc.

Product Information

Cosmid Construction Kit-3

Research use only

Catalog No: R-1003
Lot No: 3003
Expiry: 2028-04

Contents:

Reagent	Cat #	Cap Color
SfiI enzyme, 30 µL	R-1008	blue
10x SfiI buffer, 200 µL	R-1009	white
OD101 electro-competent cells, 2 x 20 µL	R-1024	yellow
Outgrowth medium for electro-competent cells, 3 x 1 mL	R-1025	green
Amp/Kan antibiotic mix, 800 µL	R-1007	mauve

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 via **electroporation**.

♦ The SfiI enzyme and its buffer are used to digest the 4 adenovirus shuttle plasmids.
♦ Electro-competent OD101 will be transformed with the products of the ligation of the SfiI DNA fragments with one another.
♦ The outgrowth medium is used to culture the cells immediately after the electric shock.
♦ The Amp/Kan antibiotic mix is used to select clones on LB/agar.

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

Quick protocol

SfiI Digestion
- Digest the 4 shuttle plasmids (5 µg each) with SfiI for 1 hr at 50°C.
- Purify the SfiI fragments of interest on agarose gel.

Ligation
- Mix equimolar amounts of the 4 gel-purified SfiI DNA fragments in a total of 8 µL.
- Add 1 µL T4 DNA ligase + 1 µL 10x T4 DNA ligase buffer.
- Incubate for 1 hr at room temperature or overnight at 4 ˚C.

Electroporation
- To a microcentrifuge tube kept on ice, add:
  - 40 µL ice-cold ddH2O
  - 3 µL electro-competent OD101 cells
  - 1 µL ligation reaction
- Mix briefly, then transfer the contents of the tube to the bottom of a 2 mm-gap electroporation cuvette.
- Apply an electric shock according to the recommendations from the manufacturer of our device.
- Resuspend the cells in 300 µL outgrowth medium.
- Transfer the cell suspension to a sterile 10-mL tube.
- Culture for 45 min at 37 ˚C with shaking.

Clone selection
- Add 20 µL Amp/Kan antibiotic mix to the cells and immediately spread on a Petri dish containing ~ 20 mL LB/agar.
- Incubate overnight at 37°C.

Consult the AdenoQuick2.0 manual for details.

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