O.D.260 Inc.

Product Information

RightZAP2.2

Research use only

Catalog No: ZR-04
Lot No: 0411

Contents:

20 μg DNA, 0.5 μg/μL
in TE pH 7.5

Storage: -20°C

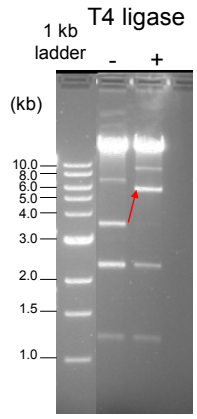
Features and Applications:
RightZAP2.2 DNA is a 29.8 kb linear DNA fragment that encompasses bp 3504-right end of the Ad5 genome. The E3 region is deleted (2.7 kb). The DNA was purified from a cosmid. It is used in combination with the shuttle vectors pZAP2.1 or pZAP2.2 to construct replication-deficient adenoviruses containing transgenes in place of the E1 region of the Ad5 genome. The maximum transgene capacity of the resulting virus is 7.9 kb.

Quality Control Assays:
Ligation:
The following reaction was set up:

2 μL RightZAP2.2
2 μL Left Arm (βGal)
4 μL H_2O
1 μL T4 DNA Ligase buffer
0.5 μL *Pac*I (10 u/μL)
0.5 μL T4 DNA Ligase

Total: **10 μL**

The reaction was performed for 1 hour at room temperature and stopped by inactivating the T4 Ligase for 10 min. at 65°C. The ligation product was digested with *Bst*1107I. This enzyme generates a 2.3 bp-long DNA fragment corresponding to the left end of RightZap2.2, and a ~3.5 kb DNA fragment corresponding to the C-terminus of β-gal left arm. In presence of T4 DNA ligase, the 3.5 kb βgal fragment is almost completely shifted to form a ~ 5.8 kb DNA fragment with the 2.3 kb DNA fragment originating from RightZAP2.2 DNA.



Virus Recovery:
The ligation mixture (2 μg DNA) was transfected into 293 cells (passage 40, 6-cm dishes) using the calcium-phosphate method. The cell monolayer was covered with agar 1 day after transfection and plaques were counted. The first plaques were visible 4 days after transfection. Nine virus clones were amplified, their genomes were purified and analyzed by restriction with *Hin*dIII. All clones presented the expected restriction pattern.