O.D.260 Inc.

Product Information

RightZAP1.4

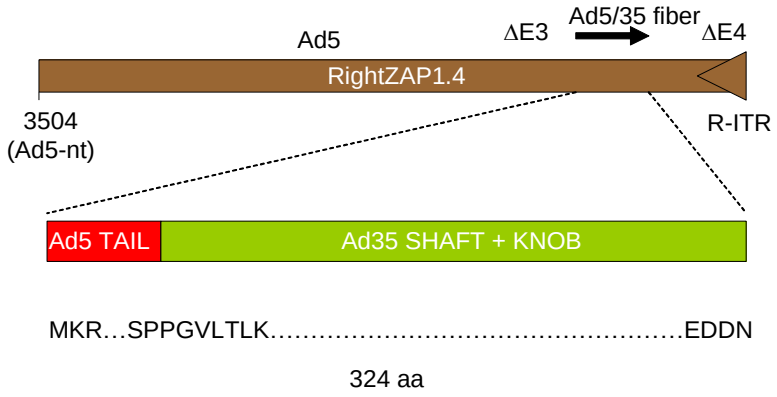
Research use only

Catalog No: ZR-06
Lot No: 0208

Contents:
20 μg DNA, 0.5 μg/μL
in TE pH 7.5

Storage: -70°C

Features and Applications:
RightZAP1.4 DNA is a 27.8 kb linear DNA fragment that encompasses bp 3504-right end of the Ad5 genome. The E3 and E4 regions are deleted (2.7 kb and 1.2 kb, respectively). The fiber is a hybrid Ad5/Ad35. It contains the N-terminal tail of Ad5 fused to the shaft and knob of Ad35. The DNA was purified from a cosmid. It is used in combination with the shuttle vector pZAP1.1 to construct replication-deficient adenoviruses containing transgenes in place of the E1 region of the Ad5 genome, and the fiber shaft and knob from Ad35. The maximum transgene capacity of the resulting virus is 9.5 kb.
Ad35 belongs to the adenovirus subgroup B that uses almost exclusively CD46 as the primary attachment receptor. CD46 is a membrane protein that is expressed ubiquitously, has complement regulatory functions, and is upregulated in tumor and stem cells.



Quality Control Assays:

Ligation:

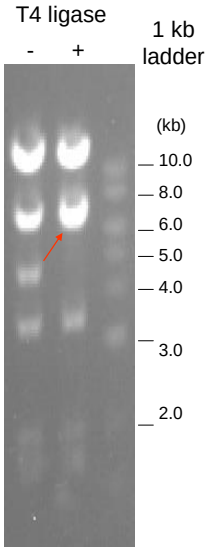
A "Left Arm" containing a β-galactosidase expression cassette was purified as a *Pac*I-*Bst*API fragment.

The following reaction was set up:

4 μL RightZAP1.4 (2 μg)
6 μL Left Arm (~ 300 ng)
6 μL H_2O
2 μL T4 DNA Ligase buffer
1 μL *Pac*I (10 u/μL)
1 μL T4 DNA Ligase

Total: **20 μL**

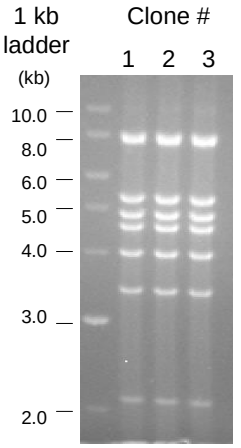
The reaction was performed for 3 hours at room temperature and stopped by inactivating the T4 Ligase for 10 min. at 65°C. The ligation product was digested with *Bst*EII. In presence of T4 DNA ligase, the 4.6 kb fragment containing the βGal cassette is almost completely shifted to form a 6.3 kb DNA fragment with the 1.7 kb *Bst*EII DNA fragment corresponding to the left end of RightZAP1.4.



Virus Recovery:

The ligation mixture (2 μg DNA) was transfected into 293 cells (passage 39, 6-cm dishes) using the calcium-phosphate method. Three days after the transfection, the cells were split. The first plaques were visible 7 days after transfection.

Three virus clones were amplified. Their genomic DNAs were purified and analyzed by restriction digestion with HindIII. All 3 clones showed the expected restriction pattern.



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