

Certificate of Analysis**pAd329**

Research use only

Catalog No: OP-02**Lot No:** 0305**Contents:**

pAd329 plasmid DNA (20 µg, Ethanol-precipitated)

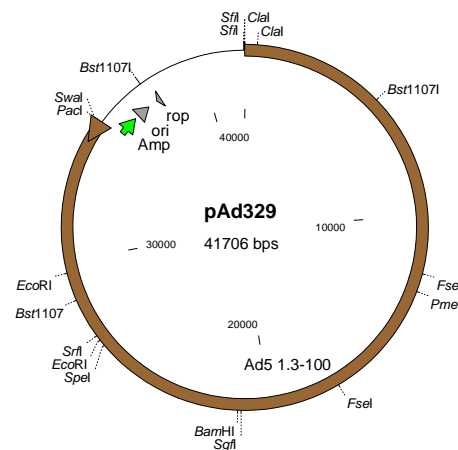
Reconstitution: The DNA precipitate was clearly visible in the tube before shipment. Make sure that you can see it. Spin the tube for 15 min. at 13,000 rpm in a microcentrifuge. Remove the supernatant carefully. Spin briefly again and remove any trace of EtOH. Leave the tube open on the bench for a few minutes in order to let the EtOH evaporate. Do not use a Spin-Vacuum device, because it will be difficult to

resuspend the DNA if it is dried too much. Add 40 µL TE pH 7.5 to the pellet, in order to obtain a concentration of approximately 0.5 µg/µL. We do not advise to resuspend in smaller volumes, since the pellet will be more difficult to resuspend at higher concentrations.

Storage after reconstitution: -20°C.

Please note that large DNAs such as pAd329 will aggregate into a viscous mass after repeated freeze-thaw. Make sure you homogenize the DNA solution each time you thaw it, by flicking the tube with your fingers, or pipetting in/out the solution slowly and carefully.

Features and Applications: pAd329 is a 41.7 kb plasmid that contains the sequences encompassing bp 466-right end (1.3–100 mu) of the Ad5 genome. The two *SfiI* sites naturally present in WT Ad5 DNA were mutated by substituting A for G and C at positions 16291 and 16294 in the Ad5 genome, and C and G for respectively G and C at positions 23001 and 23004 in the Ad5



genome, introducing silent mutations in the adenovirus pVII and DNA-binding protein coding sequences. A pair of *SfiI* sites that allow for directional cloning replaces the E1 promoter. The E3 region is intact. The right ITR is flanked by *PacI* and *SwaI* sites. pAd329 is used in combination with the shuttle vector pE1.1 to construct conditionally

replicative adenoviruses (CrAds) containing a heterologous promoter in front of the E1a TATA box. The maximum capacity for insertion is 2.1 kb.

Selection:

Prokaryotic – ampicillin 50 µg/mL

Replication:

Prokaryotic – pBR322 ori

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