P1156—HiPure Plasmid EF Maxi Kit

Additional Protocol: Further purification of plasmid DNA with isopropanol (Removal of RNA contamination)

- Column method plasmid DNA extraction kit using isopropanol or ethanol mediated binding, such as P1156 and other products, usually contains a certain amount of RNA contamination in the obtained plasmid DNA, especially in medium/low copy vectors and YT/TB cultured medium. When measuring with a UV spectrophotometer, the nucleic acid concentration does not match the brightness of the electrophoresis band, and the OD concentration is much higher than the brightness of the electrophoresis band. This is because there is degraded RNA contamination (25-100nt) in the obtained plasmid DNA. Due to the degradation of RNA bands into small molecules, the efficiency of EB/staining is low, and in most cases, the gel image does not show the degraded RNA bands.

- This protocol uses isopropanol (0.7 times) for re-precipitation/concentration, which not only increases plasmid concentration but also significantly reduces RNA contamination, making OD concentration more consistent with electrophoresis brightness.

- This protocol can also use Triton X-114, a classic endotoxin removal reagent, to further extract endotoxins, which can significantly degrade endotoxin levels, meeting the requirements for injection and transfection of sensitive cells.

1. Add purified plasmid DNA into a 2ml centrifuge tube, add ultrapure water to 1.0ml, add 0.1ml of 3M sodium acetate, pH 5.5 (or 5M NaCl, or Buffer LN3), invert and mix 3-5 times.

2. (Triton X-114 endotoxin extraction) Add 0.1ml Buffer ER2 to the plasmid solution, invert and mix 10-15 times. Place on ice or in the refrigerator (2-8°C) for 10-15 minutes, then invert and mix several times. Water bath at 42-50°C for 5 minutes. Centrifuge at 13,000 x g for 5 minutes at room temperature. Transfer the supernatant to a new centrifuge tube (high-speed centrifuge tube).

   After water bath at 42-50°C, Buffer ER2 binds with endotoxins to form a vacuole structure that is insoluble in water. After centrifugation, it forms a red solution layer at the bottom of the centrifuge tube. If no stratification is formed after centrifugation, invert and mix 5-6 times, repeat the centrifugation operation, and ensure that the centrifuge is at room temperature or has fully recovered to room temperature. Gently remove the centrifuge tube. The density of

droplet formed by Buffer ER2 and endotoxin molecules is only slightly higher than that of the supernatant, and it is easily affected by external forces to stratify. If there is a small amount of liquid droplets suspended in the supernatant, place for 3-5 minutes before transferring the supernatant. Transferring a small amount of ER2 droplets does not affect DNA production. If downstream applications are highly sensitive, it is recommended to repeat the extraction process.

3. **(Add 0.7 times the volume of isopropanol to the supernatant**, invert and mix 20-30 times, centrifuge at 13,000 x g for 15 minutes at room temperature, and carefully discard the supernatant.

   Be careful when discarding the supernatant to avoid losing the DNA precipitate. After centrifugation, DNA precipitates may not be visible, especially when dealing with low or medium copy number vectors, DNA precipitates are even less visible due to low DNA yield. For the convenience of observation, a label pen can be used to mark the centrifuge tube (at the farthest end from the center of radiation) to facilitate the observation of the direction where the sediment is located. Due to the influence of the centrifuge angle, plasmid DNA may uniformly adhere to the farthest wall of the tube, from the bottom of the tube to the liquid surface.

4. **Add 1.0ml of 70% ethanol to the precipitate.** Vortex for 5 seconds, then invert and mix several times. Centrifuge at 13,000 x g for 3 minutes, carefully discard the supernatant.

5. Collect the residual liquid by centrifugation briefly, carefully adsorb and discard all residual liquid, open the lid and dry in air for 10 minutes.

6. **Add an appropriate amount of sterile water or endotoxin free water (self-prepared) to the plasmid, gently shake to resuspend the precipitate**. Place at room temperature for 10 minutes to fully dissolve the plasmid, during which invert and mix several times. Store the plasmid at -20°C.