

Plasmid Endotoxin Removal Kit

Catalog Number	Description	Size
EB01-01	Plasmid Endotoxin Removal Kit	20 ml EndoClean solution 50 ml Elution Buffer

Endotoxin Removal Procedure

(This protocol is designed to remove endotoxin after the plasmid DNA is purified)

1. Add **0.2 volume EndoClean Solution** to the plasmid sample in a 1.5 mL sterile microtube. (For example, add 0.2 mL EndoClean Solution to 1 mL plasmid sample).
2. Mix by vortexing the tube a few times and put on ice for about 10 min until the solution is clear without turbidity. (Rocking the sample in a cold room for 10 min is recommend if it is available).
3. Mix well again by inverting the tube a few times.
4. Incubate the tube at 55 °C water bath for about 5 min and the solution shall be turbid. Add **1/25 volume chloroform** and mix well by vortexing for 20 s.
5. Centrifuge at 12,000 rpm at room temperature for 5 min with the brake off.
6. Carefully transfer the upper clear layer solution to another 1.5 mL microtube.
7. Precipitate as one of the following methods:
 - a. Add **0.1 volume 3 M NaOAc or KOAc (pH 5.2)** and **0.7 volume isopropanol**.
 - b. Add **0.1 volume 3 M NaOAc or KOAc (pH 5.2)** and **2.5 volume cold absolute ethanol (96-100%)**. Let stand in -80°C freezer for at least one hour.
8. Centrifuge sample for 15 min at highest speed in a 4°C microcentrifuge. Remove the supernatant.
9. Add **500 µL cold 70% ethanol**. Centrifuge for 5 min in a 4°C microcentrifuge. Remove the supernatant. Air-dry for 5-10 min.
10. Resuspend pellet in desired volume of **Elution Buffer** or **EndoFree H₂O**. *Note: Plasmid DNA will be kept in supercoil form better in Elution buffer than in EndoFree H₂O.*

(This protocol can be scaled up accordingly)