Instructions for Endotoxin Remover

Cat: E1040
Size: 20mL/100mL
Storage: RT, Valid for 1 year.

Introduction:

The endotoxin scavenger produced by our company is mainly used to remove endotoxins from DNA, protein or other liquid samples. Under the specific pH value, salt concentration and temperature conditions, endotoxin scavenger can specifically bind with DNA, recombinant protein and endotoxin in liquid samples. After high-speed centrifuge at room temperature, DNA or protein is retained in the water phase, while endotoxin is concentrated to a very small volume of the lower phase and removed. After more than 3 times of repeated extraction, the activity of 5000~50000EU/mL endotoxin level can be reduced to 5~0.5EU/mL below, that is, reduced 1000~10000 times.

Protocols:

1. Before extraction, please put the endotoxin remover in an ice bath for 5min, during which time turn the bottle several times to evenly pre-cool the reagent.

(1) Purified plasmid DNA endotoxin removal: 500μL DNA solution was absorbed into a microcentrifuge tube, and 1/10 volume 3M NaAc pH5.2 or 1/20 volume 5M NaCl solution was added into the ice bath for 5min.

(2) Removal of endotoxin during the extraction of plasmid DNA: Taking the extraction of plasmid by alkaline cleavage method as an example, the supernatant containing plasmid DNA was absorbed into a new centrifuge tube after the lysate and neutralization solution were added and the debris was removed by centrifugation, and the supernatant was soaked in ice bath for 5min.

2. Add 1/5 volume of pre-cooled endotoxin scavenger, shake and mix well, and the solution becomes turbid.
3. Ice bath 5min, the solution strain clear.
4. 37℃ water bath 5min, oscillating from time to time, the solution becomes cloudy.
5. Centrifuge at 12000rpm for 5min at room temperature, the solution should be divided into two phases, the upper water phase contains DNA, the lower oil phase contains endotoxin.
6. Transfer the upper water phase containing DNA to the new tube, discard the oil phase, and repeat extraction three times, that is, repeat steps 2-6 three times.
7. Add 2.5 volumes of anhydrous ethanol and precipitate at -20℃ for 30min or overnight; Centrifuge at 12000rpm for 10min, discard the supernatant; Add 70% ethanol to wash and precipitate, centrifuge at 12000rpm for 5min and discard the supernatant; Air drying precipitation, adding 100~200μL of high purity water without endotoxin or TE dissolution precipitation.
8. Use endotoxin detection reagent to determine the endotoxin activity in the sample, and compare with the initial sample.

Note:

1. DNA concentration >1mg/mL when the removal of endotoxin efficiency is reduced. Due to the nature of DNA and proteins themselves, removal procedures can result in loss of 10-20% of DNA. Fortunately, DNA is easier to extract and prepare than endotoxin removal is difficult.
2. All solutions should be prepared with high purity water without endotoxin, all equipment materials should be free of endotoxin, glassware can be baked at high temperature, and non-volatile aqueous solution can be treated at high pressure 120℃.

Related Products:

D1140 Deendotoxin plasmid small amount extraction kit
D1150 Large amount extraction kit for deendotoxin plasmid
12100 DMEM(H) (contains biantibody, does not contain sodium pyruvate)
24800 Universal cell freeze