untitled protocol

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Abstract

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Materials

IPTG I2481 by Gold Biotechnology

Protocol

Step 1.
Incubate BW25113/pRstA-GFP cells in 50 ml LB medium with chloromycetin (100 mg/ml), and expression was induced by adding IPTG (final concentration of 1.0 mM).

Step 2.
Harvest by centrifugation, the cells were resuspended in 12 ml Lysis/Equilibration buffer including 120 µl lysozyme (10 mg/ml),

Step 3.
Place on ice for 30 min, and then extracted by sonication method.

Step 4.
Centrifuge at 4 ℃□12000 rpm for 5 min.

Step 5.
Transfer supernatant to new 1.5 ml epp tube.

Step 6.
Incubate with His-Select Ni-NTA Agarose (Thermo Fisher Scientific, USA) over night at 4°C.

Step 7.
The next day, the supernatant was loaded onto Ni-NTA affinity resin (Qigen, Germany).

Step 8.
Proteins were examined for purity by SDS-PAGE at 200 V for 45 min. Stain gel with extant blue for 15 min.

Step 9.

The pure protein were pooled and dialyzed.

Step 10.

The extraction of proteins was analyzed by BCA protein concentration quantitative method.