ATAGENIX LABORATORIES

Catalog Number:ATO00034

Inclusion bodies Purification assay kit

Product Details

Summary

Product Name	Inclusion bodies Purification assay kit
Standard Operating Procedure	1. Collect inclusion bodies (1) Collect the bacteria by centrifugation. (2) Add 10ml of Buffer P1 (recommended volume: 1/20 of cell culture volume) to suspend bacteria (recommend to process 200ml bacteria solution at one time). (3) Ultrasound under ice bath, breaking bacteria for 8s, interval 8s, 80 times (frequency above 200Hz). Note: This step requires low temperature and rapid operation. (4) Immediately add protease inhibitor PMSF, etc. (5) Centrifuge at 10000g for 10 minutes at 4 ℃to collect inclusion bodies. 2. Purification of inclusion bodies 2.1 When the expression level of target protein is high, a large amount of soluble recombinant protein can be obtained from inclusion bodies according to the following steps Note: Please 1:10 dilute 10 × Buffer P2 with ddH2O into working buffer. (1) Inclusion bodies are resuspended with 10ml of Buffer P2 . (2) Centrifuge at 10000g for 10 min at 4 ℃ . (3) Repeat the above two steps 3-6 times. (4) Discard the supernatant and suck up the residual liquid. (5)Add 9ml ddH2O to suspend and mix the inclusion bodies. Add 1ml Buffer P3 to dissolve the inclusion bodies, mix well, and leave at room temperature for 15 minutes. (The volume of ddH2O and Buffer P3 can be adjusted according to the volume of the inclusion bodies) (6) Centrifuge at 10000g for 10 min at 4 ℃ and collect the supernatant. (7) Replace with dialysis solution (TBS buffer, PH8.0) for dialysis.

(8) Centrifuge at 10000g for 10 min at 4 ℃, and collect the supernatant, which is the target protein. If there is big amount of precipitation, add 1% Buffer P5 into dialysis solution can help to reduce protein lost.

2.2 When the expression level of target protein is low, follow these steps to obtain soluble recombinant protein from inclusion bodies with better purity (for example: Ni column affinity purification)

(1) Add 9ml ddH2O to suspend and mix the inclusion bodies. Add 1ml Buffer P3 to dissolve the inclusion bodies, mix well, and leave at room temperature for 15 minutes. (The volume of ddH2O and Buffer P3 can be adjusted according to the volume of the inclusion bodies)

(2) Centrifuge at 10000g at 4 ℃ for 10 minutes, and transfer the supernatant to a clean tube.

(3) The subsequent purification process is the same as the soluble protein purification step. Absorb protein with Ni filler, wash buffer wash off impurities and elute the buffer to obtain the protein of interest. Adding 1/10 volume of Buffer P4 to the washing buffer and eluting buffer helps to increase the yield of soluble protein.

(4) Replace with dialysis solution (TBS buffer, PH8.0) for dialysis.

(5)Centrifuge at 10000g for 10 min at 4 ℃, and collect the supernatant, which is the target protein. If there is big amount of precipitation, add 1% Buffer P5 into dialysis solution can help to reduce protein lost.

Description

Inclusion bodies refer to inactive particles formed by aggregation of overexpressed proteins in the cell cytoplasm or intermembrane. The formation of inclusion bodies is due to lack of protein folding cofactors during the expression of recombinant proteins, or the incorrect buffer results in the inability to form correct secondary bonds. Since inclusion bodies are mainly composed of overexpressed recombinant proteins, the isolation and purification of inclusion bodies is the first step to produce active recombinant proteins. The inclusion bodies purification assay kit is used for rapid purification of inclusion bodies to obtain soluble proteins, which can be used in subsequent experiments without refolding.

Advantage

The inclusion bodies purification assay kit is used for rapid purification of inclusion

	bodies to obtain soluble proteins, which can be used in subsequent experiments without refolding.
Storage	Store at room temperature for one year.
Shipping	The products are shipped out with blue ice .
Note	For research use only.