V. 1.0

FtsZ Protein

Cat. # FTZ01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material
E. coli FtsZ protein has been purified after over-expression in *E. coli.* FtsZ has an approximate molecular weight of 40 kDa (Fig. 1). FTZ01 is provided as 1 mg of lyophilized protein.

Storage and Reconstitution
The lyophilized protein when stored desiccated to < 10% humidity at 4°C is stable for 6 months. Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml by pipetting 200 ul of distilled water in each vial. The protein will then be in the following buffer: 50 mM Tris-HCl pH 8.0, 50 mM KCl, 1mM EDTA, 5% (w/v) sucrose, 1% (w/v) dextran. The concentrated protein should then be aliquoted and snap frozen in liquid nitrogen. Store the aliquots at -70°C for 6 months. For working concentrations, further dilution of the protein should be made with MES Buffer (100 mM MES pH6.5, 1mM EGTA). It is not advisable to repeatedly freeze thaw FtsZ protein.

Purity
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 10% polyacrylamide gel. FtsZ protein was found to be >90% pure (see Figure 1, Lane 1).

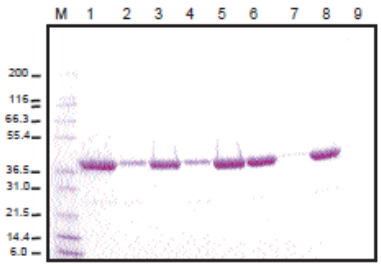

Figure 1. FtsZ Polymerization. FtsZ protein was diluted to 1 mg/ml in 100 mM MES pH 6.5, 1 mM EGTA and divided into 4 tubes. Two tubes only were supplemented with 20 mM $CaCl_2$ and 2 mM and GTP. The tubes were incuabted at 37°C for 15 minutes and centrifuged at 20,000 rpm for 20 minutes. Supernatants and pellets were examined. Lane 1,10 µg FtsZ protein, Lanes 2 & 4, supernatants from tubes supplemented with $CaCl_2$ and GTP, Lanes 3 & 5, pellets from supplemented tubes. Lanes 6 & 8, supernatants from non-supplemeted tubes, Lanes 7 & 9, pellets from non-supplemeted tubes. Lane M, Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay
The biological activity of FtsZ can be determined in two ways, first from its ability to efficiently polymerize into filaments *in vitro* in the presence of Ca^{2+} and GTP, and secondly to hydrolyze GTP to GDP and Pi. Polymerization by Ca^{2+}:GTP results in bundles of FtsZ filaments that sediment at 50,000xg, analysis of the pellet and sup by protein assay or SDS-PAGE indicates the proportion of polymerizable FtsZ protein, the Batch of protein passes Quality Control when greater than 70% of FtsZ is in the pellet. GTPase activity is determined by measuring the Pi released during a 5min reaction, by using the malachite green reagent Cat# BK050. The Batch of protein passes Quality Control when a specific activity greater than 0.3mol GTP / mol FtsZ / min is obtained.

Reagents
1. FtsZ Protein (Cat.# FTZ01)
2. MES Buffer (100mM MES pH6.5, 1mM EGTA)
3. GTP Stock 100mM (Cat.# BST06)
4. Calcium chloride solution 200mM
5. Precision Red Advanced Protein Assay Reagent (Cat.# ADV02)
6. CytoPhos Assay Reagents (Cat.# BK054)

Equipment
Microfuge at 4°C
Airfuge or Ultracentrifuge (capable of 50,000 x g)
Ultracentrifuge tubes (Beckman Cat. # 344718)
Spectrophotometer with 600nm (ADV02) capability.

Method

Polymerization Assay
1. Resuspend 1mg FTZ01 to 1.0 mg/ml in MES buffer and place vial on ice.
2. Incubate on ice for 30 min to fully resuspend the protein.
3. Centrifuge the protein in a 4°C microfuge at 14K rpm for 15 min.
4. Transfer the supernatant to a new microfuge tube and determine the total protein concentration with the Precision Red Advanced Protein Assay Reagent (Cat# ADV02).
5. Aliquot 200 ul of the FtsZ solution into two ultracentrifuge tubes.
6. Add 4ul of GTP into each tube and mix well.
7. Add 20 ul (1/10th the volume) of 200mM calcium chloride solution into each tube and mix well.
8. Incubate at 37°C for 15min.
9. Centrifuge the tubes at 50,000 x g for 20min to pellet the polymerized FtsZ.
10. Pipette off the supernatant of each tube into a clean micro-

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

fuge tube.

11. Analyse samples by SDS-PAGE and visualize FtsZ using coomassie blue stain. The addition of calcium and GTP allows the FtsZ to polymerize and results in <70% of protein appearing in the pellet. FtsZ samples that are not supplemeted with Ca^{2+}:GTP will have >70% of FtsZ in the supernatant (see Fig. 1).

GTPase Assay

Equipment and material required

1. Spectrophotometer capable of measuring absorbance at 650nm wavelength.
2. Small capacity (100-1000ul) cuvettes or 96-well microtitre plates.
3. Pipettors 20,200 and 1000ul capacity.
4. 10mM GTP in Milli-Q (nanopure) water.
5. 37°C waterbath.

Protocol:

1. Resuspend 1mg FTZ01 to 1.0 mg/ml in 100 mM MES pH 6.5, 5 mM Mg acetate, 2 mM $CaCl_2$ and 1 mM EGTA (1ml).
2. Incubate on ice for 5 min to fully resuspend the protein.
3. Optional: determine the total protein concentration with the Precision Red Advanced Protein Assay Reagent (Cat# ADV02) or your own laboratory standard protein assay.
4. Label five tubes with T=0, 5, 10, 15 and 20min and place on ice.
5. Pipette 100 ul FtsZ into a 1.5ml reaction tube and place on ice.
6. Pipette 5ul of 10mM GTP into the reaction tube and mix by vortexing 5 seconds.
7. Immediately place the reaction tube in the 37°C waterbath.
8. Immediately take the first sample (T=0min) by pipetting 15ul into the T=0 tube and immediately freeze the contents in a liquid nitrogen bath or -70C freezer.
9. Repeat with 15ul samples every 5min, pipetting into the appropriate labeled tube.
10. When all the samples have been collected, defrost them at the same time by placing in a room temperature waterbath for exactly 15 seconds.
11. Immediately pipette 100ul of Cytophos reagent (Cat.# BK054) into each tube, incubate for 10min and read absorbance in a 96-well spectrophotometer. Calculate specific activity in mols Pi / mol FtsZ / min.

Note: It is not possible to quench reactions on ice or with EDTA. If HTS through-put is required we suggest performing the assay with two time points (T=0 and T=20min) and processing all samples from the same time point at the same time.

Product Uses

- Identification and characterization of FtsZ binding proteins
- Characterization of FtsZ dynamics
- Developing FtsZ ligands that may be used as anti-bacterial agents.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.