V. 3.0

FtsZ protein (>90% pure)
Source: *Enterococcus faecalis* FtsZ expressed in *E.coli*
Cat. # FTZ04

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

Background Information

The tubulin homolog FtsZ protein (Filamenting temperature-sensitive mutant Z) is essential for bacterial cell division and an ideal target for novel anti-bacterial drugs (1). Mutants lacking this protein do not divide, but continue to elongate into filaments. FtsZ is a GTPase that polymerizes in a nucleotide-dependent manner head-to-tail to form single-stranded proto-filaments. Proto-filaments assemble into a contractile ring termed the Z-ring which forms on the inside of the cytoplasmic membrane and marks the future site of the septum of a dividing bacterial cell. Although FtsZ polymerization rapidly reaches steady state, the Z-ring is dynamically maintained through the course of cell division by continuous and rapid turnover of FtsZ polymers, likely fueled by FtsZ's GTP hydrolysis (2-4).

FtsZ proteins from different species are highly divergent (40 to 70% homologous) compared to eukaryotic tubulins (90-99%). In practical terms this manifests itself in two important ways, first drugs that are developed to one FtsZ protein as a general rule will not bind and inhibit FtsZ function from another species (e.g. ref. 6). Secondly, the buffer conditions for optimal GTPase activity can be vastly different for different species FtsZ proteins. *In vitro* FtsZ assembles into proto-filaments, two-dimensional sheets, and proto-filament rings (1-5). Interestingly, the buffer optima for protofilament related GTPase activity is different from that which produces sedimentable sheets and filaments. For example *Enterococcus faecalis* FtsZ protofilament GTPase is highly active in Buffer 2 (see high ratio in Table 1) whereas sheets and filaments are best formed in Buffer 4 (see Biological Activity section). For a full description of FtsZ buffer preferences visit www.cytoskeleton.com.

Table 1: GTPase optimization table for *Enterococcus faecalis* FtsZ

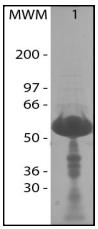
Activity*	Buffer***			
	1	2	3	4
GTPase	5.8	10	16	16
ATPase	2.3	0.7	1.9	2.2
Ratio GTPase/ATPase	2.5	14	8.2	7.4
Critical Concentration**	nd	nd	nd	0.2

* Activity in nmole GTP / mg FtsZ / min.
** CC for Sheets and filaments measured in mg / ml.
*** **Buffer 1**: 50mM MES-KOH pH 6.5, 50mM KCl, 5mM $MgCl_2$.
Buffer 2: 50mM Hepes-KOH pH 6.8, 250mM KCl, 5mM Mg acetate.
Buffer 3: 50mM Hepes-KOH pH 7.5, 300mM K-acetate, 5mM Mg acetate.
Buffer 4: 50mM Hepes-KOH pH 7.5, 100mM K-glutamate, 300mM K-acetate, 5mM Mg-acetate, 20% Ficoll 70K.

Material

Recombinant *Enterococcus faecalis* FtsZ protein has been purified after over-expression in *E.coli*. The protein has a 6xHis C-terminal tag and an approximate molecular weight of 52 kDa (Figure 1). FTZ02 is supplied as a white solid. The protein purity was determined to be >90% (Figure 1).

Figure 1: Purity Analysis of *E. faecalis* FtsZ Protein



Legend: A 100 µg sample of *E. faecalis* FtsZ protein was separated on a 4-20% gradient SDS-PAGE gel and stained with Coomassie blue. Lane 1, 100µg FTZ04. SeeBlue molecular weight markers are from Invitrogen.

Storage and Reconstitution

The recommended storage conditions for the lyophilized material is desiccated at 4°C. Under these conditions the protein is stable for 1 year. Lyophilized protein can also be stored desiccated at -70°C.

Reconstitute to 5 mg/ml with distilled water (200 µl water per mg FtsZ). Incubate on ice for 10 minutes to fully resuspend the protein. The protein will then be in the following buffer: 10 mM Hepes-KOH pH 7.5, 50 mM KCl, 10µM GDP, 5% (w/v) sucrose, 1% (w/v) dextran. The concentrated protein should be aliquoted, snap frozen in liquid nitrogen, and stored at -70°C (stable for 6 months). For working concentrations, further dilution of the protein should be made with the recommended buffer (see Table 1 and Assay methods section). Frozen aliquots should be defrosted rapidly by placing in a room temperature water bath, it is not advisable to repeatedly freeze thaw the protein.

Biological Activity Assay

The biological activity of FTZ04 is assessed as follows;

Sheet/Filament Sedimentation Assay: FtsZ from *E. faecalis* can polymerize into protofilaments and sheets *in vitro* in the presence of Mg^{2+} and GTP. Polymerization and sheet formation is enhanced at physiological pH, potassium and acetate rich buffers and a crowding reagent such as that in Buffer 4 (Table 1) which results in polymers that form a turbid solution and sediment at 100,000xg. Analysis of the pellet and supernatant by protein assay or SDS-PAGE indicates the proportion of polymerizable FtsZ protein. Under the experimental conditions described, GTP-dependent pelleted protein is >65% of total protein, and supernatant protein is in agreement with its known critical concentration for assembly (0.2mg/ml at 37°C, in Buffer 4).

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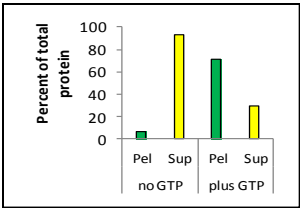
GTPase Activity Assay: The GTPase activity of *E. faecalis* FtsZ is determined in Buffer 2 (Table 1) by measuring phosphate ions released kinetically using a MESG phosphate detection kit (Cat. # BK052) or an endpoint assay (Cat. # BK054). Under the experimental conditions described, a specific activity greater than 8 nmole / mg / min is obtained.

Sheet /filament Sedimentation Assay Method

1. Resuspend FTZ04 to 5 mg/ml as detailed in Storage and Reconstitution section.
2. Dilute the protein to 1.0 mg/ml in Buffer 4 (4 µl of Buffer 4 per 1 µl of FTZ04).
3. Prepare two ultra centrifuge tubes labeled 1 and 2.
4. Pipette 10µl of Milli-Q water into tube 1 (Monomer control), and 10µl of 10mM GTP into tube 2 (Sheet/filament test).
5. Pipette 100 µl of the FtsZ solution into each tube.
6. Incubate at 37°C for 10min.
7. Centrifuge the tubes at 100,000 x g for 30min to pellet the polymerized FtsZ.
8. Pipette off the supernatant of each tube into a clean microfuge tube.
9. Analyze samples by Protein Assay (e.g. ADV02). Less than 10% of protein should appear in the pellet of Tube 1, and greater than >65% of protein should appear in the pellet of Tube 2 (Figure 2).

Figure 2: Example sheet/filament sedimentation results

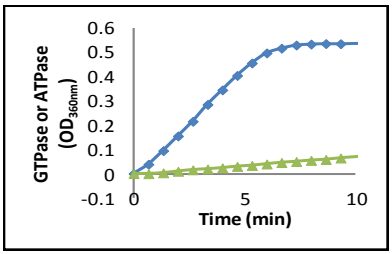


Legend: Sample were prepared as described in the sedimentation protocol and after centrifugation the samples were assayed for protein with ADV02 protein assay.

Protofilament GTPase Assay Method

1. Resuspend FTZ04 to 5 mg/ml as detailed in Storage and Reconstitution section.
2. Dilute the protein to 1.5 mg/ml in ice cold Buffer 2 (2.3 µl of Buffer 2 per 1 µl of FTZ04).
3. At room temperature, create FtsZ mix: 100µl FtsZ, 20 µl of 1mM 2-amino-6-mercapto-7-methylpurine riboside (MSEG, see Cat. # BK052) and 1.2µl of purine nucleoside phosphorylase (PNP, see Cat. # BK052). PNP is at 0.1 U per µl: one unit of PNP will cause the phosphorolysis of 1 µmole of inosine to hypoxanthine and ribose 1-phosphate per minute at pH 7.4 at 25°C.
4. Pipette 12 µl 12mM GTP into 2 wells of a 1/2 area plate (Corning Cat. # 3696).
5. Pipette 12 µl of 12mM ATP into 2 wells of a 1/2 area plate.
6. Pipette 96 µl of FtsZ mix into all four wells.
7. Place plate in a pre-warmed microplate reader at 37°C and read at 360 nm for 30min, one reading every 30 seconds (Figure 3).
8. Calculate specific activity in nmols Pi / mg FtsZ / min.

Figure 3: Example *E. faecalis* FtsZ GTPase data



Legend: GTPase reactions were carried out as indicated in the Method. Under these conditions an OD360 of 1.0 / cm is equal to 250µM of GTP hydrolyzed. There are two phases of GTPase, first polymerization which is usually complete within 3 min for the control (blue line), and then Phase II is a steady state phase where subunits and GTPase activity turns over at a certain rate without affecting the polymer mass. Duplicate reactions were performed. ATPase is depicted in the green line.

Important Technical Notes when Working with FtsZ protein

1. FtsZ proteins are highly sensitive to buffer components, a simple change from acetate to chloride can abolish GTPase activity. Use only the recommended buffer unless other experimental conditions override them.
2. For more information about FtsZ please visit the www.cytoskeleton.com .

Product Uses

- IC50 & EC50 determinations for anti-bacterial drug lead-compounds.
- Characterization of FtsZ binding proteins.

References

1. Erickson et al (1996). Proc. Natl. Acad. Sci. USA. 93, 519-523.
2. Erickson et al (2010). Mol. Biol. Rev. 74, 504-528.
3. Mingorance et al (2010). Trends Microbiol. 18, 348–56.
4. Osawa and Erickson (2006). J. Bacteriol. 188, 7132-7140.
5. Erickson (1997). Trends Cell Biol. 7, 362-367.
6. Haydon et al (2008). Science. 321, 1673-1675.

Product Citations/Related Products

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