V. 10.0

FtsZ protein (>90% pure)
Source: *Staphylococcus aureus* FtsZ expressed in *E.coli*.
Cat. # FTZ02

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 GTPase activity is different from that which produces quantifiable polymerization based turbidity. For example Staphylococcus aureus FtsZ protofilament GTPase is highly active in Buffer 3 (see Table 1) whereas polymerization is best assayed in Buffer 5 (see Biological Activity section). For a full description of FtsZ buffer preferences visit www.cytoskeleton.com.

Table 1: GTPase optimization table for Staphylococcus aureus FtsZ.

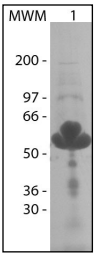
Activity	Buffer***				
	1	2	3	4	5
GTPase*	0	0	7.5	0	nd
ATPase	0	0	1.5	0	nd
Ratio GTPase/ATPase	na	na	5.0	na	na
Polymerization (V_{max})**	nd	nd	nd	nd	136

* Activity in nmole GTP/mg FtsZ/ min.
** milliOD units/min.
*** **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.
Buffer 5: 50mM PIPES-KOH pH 6.8, 250mM KCl, 5mM Mg acetate.

Material

Recombinant *Staphyloccus aureus* 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 55 kDa (Figure 1). FTZ02 is supplied as a white solid. The protein purity was determined to be >85% (Figure 1).

Figure 1: Purity Analysis of *S. aureus* FtsZ Protein



Legend: A 100 µg sample of *S.aureus* FtsZ protein was separated on a 4-20% gradient SDS-PAGE gel and stained with Coomassie blue. Lane 1, 100µg FTZ02. 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, 400 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 and thaw the protein.

Biological Activity Assay

The biological activity of FTZ02 is assessed as follows;

Polymerization Assay: FtsZ from *S. aureus* can polymerize into protofilaments and sheets *in vitro* in the presence of Mg^{2+} and GTP. GTP-dependent *S. aureus* FtsZ polymerization is optimal in Buffer 5 (Table 1), which results in polymers that form a turbid solution that can be monitored by kinetic absorbance measurements at 360nm.

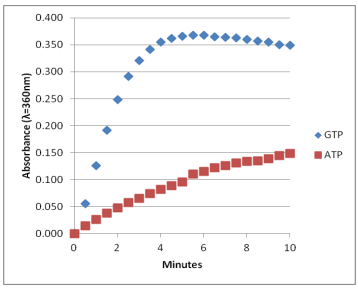
GTPase Activity Assay: The GTPase activity of FtsZ is determined in Buffer 3 (Table 1) by measuring inorganic phosphate released kinetically using a MESG phosphate detection kit (Cat. # BK052) or an endpoint assay (Cat. # BK054). Under the experi-

mental conditions described, a specific activity greater than 5 nmole / min /mg is obtained.

Polyrmerization Assay Method

1. Resuspend FTZ02 to 5 mg/ml as detailed in Storage and Reconstitution section.
2. At room temperature, create FtsZ mix: 100µl FtsZ (5 mg/ml), 125µl MilliQ water, and 250µl 2x concentrated Buffer 5.
3. Pipette 5µl 10mM GTP into 2 wells of a 1/2 area plate (Corning Cat. # 3696).
4. Pipette 5µl of 10mM ATP into 2 other wells of the 1/2 area plate.
5. Pipette 95 µl of FtsZ mix into all four wells.
6. Place plate in a pre-warmed microplate reader at 37°C and read at 360 nm for 30min, one reading every 30 seconds (Figure 2).

Figure 2: Example *S. aureus* FtsZ polymerization assay results

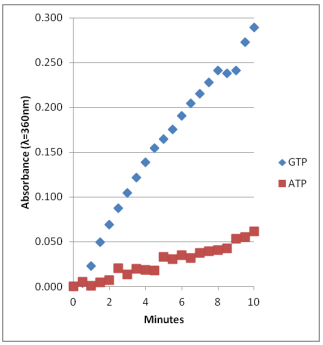


Legend: S. aureus FtsZ polymerization assay performed as described in the above method. Data set in blue represent reactions containing 0.5mM GTP and those in red represent the assay performed with 0.5mM ATP. Data shown is an average of triplicate reactions.

GTPase Assay Method

1. Resuspend FTZ02 to 5 mg/ml as detailed in Storage and Reconstitution section.
2. At room temperature, create FtsZ mix: 100µl FtsZ (5 mg/ml), 20µl MilliQ water, 250µl 2x concentrated Buffer 3, 100 µl of 1mM 2-amino-6-mercapto-7-methylpurine riboside (MESG, see Cat. # BK052) and 5µl 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.
3. Pipette 5µl 10mM GTP into 2 wells of a 1/2 area plate (Corning Cat. # 3696).
4. Pipette 5µl of 10mM ATP into 2 wells of a 1/2 area plate.
5. Pipette 95 µl of FtsZ mix into all six wells.
6. 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).
7. Calculate specific activity in nmols Pi / mg FtsZ / min.

Figure 3: Example *S. aureus* 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 200µM of GTP hydrolyzed. GTPase assay data are indicated in blue and ATPase reactions are shown in green. Data shown is an average of triplicate reactions.

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 *S. aureus* FtsZ binding proteins.

References

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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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