ProductInformation

Anti-GroES
Developed in Rabbit
IgG Fraction of Antiserum

Product No. **G8909**
Lot 085H4840

Anti-GroES is developed in rabbit using repeated injections of recombinant GroES (chaperonin 10) conjugated to KLH as the immunogen. Whole antiserum is fractionated and then further purified by ion exchange chromatography to provide the IgG fraction of antiserum. This fraction is essentially free of other rabbit serum proteins. Rabbit Anti-GroES is provided as a liquid in 0.01 M phosphate buffered saline, pH 7.4, with 0.1% sodium azide (see MSDS)* as a preservative.

Specificity
Rabbit Anti-GroES is specific for GroES by immunoblotting using a heat shocked *E. coli* extract and dot blot immunoassay using recombinant GroES.

Description
GroES, also known as chaperonin 10 (cpn10), is a highly characterized member of a class of ubiquitous and conserved *E. coli* proteins known as chaperonins. The chaperonins are a ubiquitous and indispensable family of proteins which facilitate protein folding in an ATP-dependent manner enhancing the yield of properly folded substrate proteins under conditions where spontaneous folding does not occur. GroES is a homo-heptamer protein composed of 10 kD subunits forming a ring[1]. It cooperates with another member of the chaperonin family, GroEL, which is a tetradecamer (14-subunit) protein of 58 kD subunits with a K^+ dependent ATPase activity. The active GroEL protein consists of two stacked rings of seven subunits each [2]. One ring of GroES binds to two rings of GroEL. This occurs only in the presence of adenine nucleotides and creates an asymmetric complex of $GroES_7$*ADP_7 *$GroEL_7$-$GroEL_7$.[3] Symmetric structures consisting of two rings each of GroEL and GroES have been identified as well.[4] Formation of symmetric complexes follows the binding of ATP, whereas the dissociation of one ring of GroES follows ATP hydrolysis. The current model suggests that the folding reaction by GroEL is driven by cycles of binding and release of the co-chaperone GroES.[5] These alternate with binding and release of unfolded protein substrate. The cycles result from the opposite effects of GroES and unfolded protein on the GroEL complex. The former stabilizes the ADP-bound state of GroEL, whereas the latter stimulates ADP-ATP exchange. The substrate protein goes through multiple cycles of binding and release, and is released into the cavity of the GroEL complex where it can undergo folding without interacting with the nearby folding intermediates.

Protein Concentration: 19.6 mg/ml by $E_{280}^{1\%}$ = 14.0.

Working Dilutions
1. Dot Blot Immunoassay
   a. A dilution of 1:500 detected recombinant GroES immobilized on nitrocellulose membranes (100 ng/dot). No reactivity is observed using recombinant GroEL (100 ng/dot).
   b. A dilution of 1:5,000 was determined in an indirect chemiluminescence assay using 50 ng recombinant GroES/dot. Luminol plus enhancer was used as substrate.

2. Indirect Immunoblotting
   a. A dilution of 1:200 was determined using a heat shocked *E. coli* cell extract.
   b. A dilution of 1:7,500 was determinded by indirect chemiluminescence using a heat shocked *E. coli* cell extract.

3. Ouchterlony Double Diffusion
   In 1% agarose, 5 µl of serially diluted reconstituted antiserum is reacted against 5 µl of 2 mg/ml solution of recombinant GroES (well seperation: 7.5mm center to center). A dilution of 1:8 was the highest dilution of antiserum resulting in a visible precipitate after 24 hours.

In order to obtain optimum results, it is recommended that each individual user determine their working dilutions by titration assay.

Uses
This antibody may be used to study GroES applying various immunoassays including immunoblotting, dot blot immunoassay and Ouchterlony double diffusion.

Storage
Store at –20 °C. After use, the remainder of the product may be stored as aliquots at –20 °C. Prolonged storage and repeated freezing and thawing is not recommended.

* Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

References

1. Chandrasekhar, G., et al., J. Biol. Chem., **261**, 12414 (1986).
2. Hendrix, R., J. Mol. Biol., **129**, 375 (1979).
3. Todd, M., et al., Biochemistry, **32**, 8560 (1993).
4. Schmidt, M., et al., Science, **265**, 656 (1994).
5. Martin, J., et al., Nature, **366**,228 (1993).

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