V. 1.0

Guanosine 5'-O-(3-thiotriphosphate) tetralithium salt (GTPγS)

Cat. # BS01

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

Material

Guanosine 5'-O-(3-thiotriphosphate) tetralithium salt (GTPγS) is supplied as a white powder.

Chemical formula $C_{10}H_{12}N_5O_{13}P_3S$ 4Li. The molecular weight of the trilithium salt GTPgS is 562.98. CAS # 94825-44-2, EC # 305-606-1.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The lyophilized compound, when stored desiccated to <10% humidity at 4°C is stable for 6 months. The GTPγS should be reconstituted to 20 mM with 50 µl of de-ionized water. The GTPγS should then be aliquoted into experiment sized amounts, snap frozen in liquid nitrogen and stored at or below -20°C . The GTPγS stock is stable for 6 months if stored at or below -20°C.

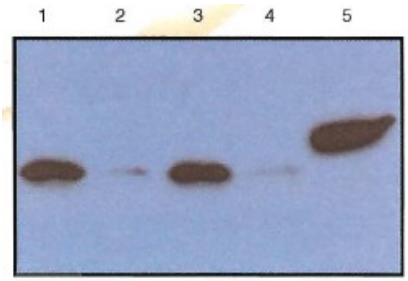
Purity

Compound is greater than 75% pure as determined by HPLC analysis.

Uses

GTPγS is a non-hydrolysable analog of GTP and is a G-protein activator. GTPγS can be used to activate small G-proteins such as Rac1 and RhoA in vitro, see Figure 1 and Biological Activity section of this profile.

Figure 1. Activation of RhoA by GTPγS Loading. Swiss 3T3 cell lysates were loaded with GTPγS (Lanes 1 & 3) or GDP (Lanes 2 & 4). Lysates (800 µg per reaction) were analysed using Rhotekin-RBS beads in a RhoA activation pull down assay (Cat. # BK036). The beads selectively bind to GTPγS (activated) RhoA protein. Lane 5 is 20 ng of recombinant RhoA-His protein. The molecular weight of endogenous RhoA is approximately 23 kD, RhoA-His runs at approximately 25 kD due to the His-tag region.



Biological Activity Assay

The biological activity of GTPγS can be determined by the ability of the compound to activate a small G-protein such as RhoA or Rac1. In this assay the GTPγS is exchanged into the nucleotide binding site of the small G-protein in vitro and the percent of GTPγS bound protein is determined by an affinity assay. The GTPγS should give greater than five fold increase in activated small G-protein when compared to a GDP bound protein.

Reagents

1. RhoA-His protein
2. Rac1 protein
3. Loading buffer (150 mM EDTA)
4. STOP buffer (600 mM $MgCl_2$)
5. GTPγS stock (20 mM)
6. GDP stock (100 mM)
7. Precision Red Protein Assay Reagent (Cat. # ADV02) for protein quantitation in lysates

Equipment

1. Microfuge at 4°C
2. Cytoskeleton RhoA activation assay kit (Cat # BK036) or RhoA G-LISA assay (Cat. # BK124)
3. Cytoskeleton Rac1 activation assay kit (Cat # BK035) or Rac1 G-LISA assay (Cat. # BK128)
4. SDS-PAGE apparatus
5. Western blot apparatus

Method

1. Place 5 ml of a clarified 1 mg/ml solution of cell lysate from 70% confluent Swiss 3T3 cells at room temperature and add Loading buffer to 15 mM final concentration.
2. Divide the lysate into 2 x 2.5 ml.
3. Add GTPγS to one tube to a final concentration of 0.2 mM.
4. Add GDP to the second tube to a final concentration of 1 mM.
5. Incubate both tubes at room temperature for 15 minutes.
6. Stop the reactions by adding STOP buffer to a final concentration of 60 mM $MgCl_2$.
7. Immediately use the lysates in a Rac1 (Cat # BK035) or RhoA (Cat # BK036) activation assay as described in the product literature. Briefly, lysates are added to affinity beads that will selectively bind the GTPγS form of the small G-protein. Bound Rac1 or RhoA are detected by western blot analysis. See Figure 1.

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References

1. Ren, X.D., Kiosses, W.B., and Schwartz, M.A. (1999) EMBO J. 18: 578.
2. Bernard, V., Bohl, B.P., Bokoch, G.M. (1999) J. Biol. Chem. 274: 13198-13204.

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