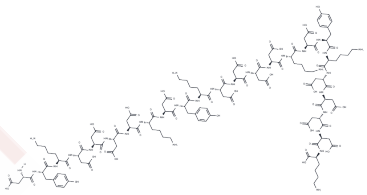
Data Sheet (Cat.No.TP2174)

TargetMol

3× FLAG peptide

Chemical Properties



CAS No. :

Formula: C123H176N30O58

Molecular Weight: 3002.88

Storage: Keep away from moisture
Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	3× FLAG peptide is a FLAG peptide tag composed of three repeated Asp-Tyr-Lys-Xaa-Xaa-Asp motifs, used for protein identification and purification.
Targets(IC50)	Others
In vitro	3× FLAG peptide is often used for elution of anti-Flag resins, magnetic beads, etc. Take the steps of purifying human histidine-tRNA synthetase (HARS) as an example:: 1. HEK293 cells transiently transfected with HARS expression plasmid were harvested and lysed in CelLytic M buffer containing mammalian protease inhibitor cocktail at 4 °C for 20 min. 2. FLAG-tagged HARS was purified by binding to anti-DYDDDDK resin and eluted by competition with 3× FLAG peptide in a buffer containing 50 mM Tris-HCl (pH 7.4) and 150 mM NaCl. 3. The isolated FLAG-HARS was further purified using HiTrapQ HP column (GE Healthcare) and eluted with NaCl gradient to 150–500 mM. 4. Fractions containing HARS were identified by SDS-PAGE, pooled and dialyzed at 4°C into a standard buffer consisting of 50 mM HEPES pH 7.5, 150 mM KCl, 10 mM MgCl2 and 5 mM β-mercaptoethanol (β-ME). 5. After dialysis, samples were concentrated using Amicon Ultra-4 centrifugal filters (Millipore) and then diluted by adding 80% glycerol to a final glycerol concentration of 40%. 6. Protein concentration was determined by A280 and stored at −20°C. The HARS content of the preparations was greater than 99% as analyzed by SDS-PAGE (data not shown). 7. The activity of each enzyme preparation was determined by active site titration26,27, monitoring the appearance of α-labeled 32P-AMP in the presence of histidine under pre-steady-state conditions with rapid chemical quenching. Prepare two syringes: in one syringe, incubate the enzyme at a concentration of 5 μM with a standard buffer containing saturated histidine, 5 mM MgCl2, and 8 U/mL pyrophosphatase (PPiase); in the other syringe, incubate ATP with a standard buffer. 8. Quench the reaction with 400 mM NaOAc (pH 4.5) and 0.1% SDS, and analyze the products by thin layer chromatography.[1]

Solubility Information

Solubility	DMSO: 23.81 mg/mL (7.93 mM),Sonication is recommended. TBS (0.5M Tris-HCl, pH 7.4, with 1M NaCl): 20 mg/mL (6.66 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	0.333 mL	1.6651 mL	3.3301 mL
5 mM	0.0666 mL	0.333 mL	0.666 mL
10 mM	0.0333 mL	0.1665 mL	0.333 mL
50 mM	0.0067 mL	0.0333 mL	0.0666 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Abbott JA, et al. The Usher Syndrome Type IIIB Histidyl-tRNA Synthetase Mutation Confers Temperature Sensitivity. Biochemistry. 2017;56(28):3619-3631.

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