

Product Data Sheet

Product Name: FLAG tag Peptide

Cat. No.: GP10150

Chemical Properties

Cas. No. 98849-88-8

Chemical Name FLAG Peptide

SMILES C1=CC(=CC=C1CC(C(=O)NC(CCCCN)C(=O)NC(CC(=O)O)C(=O)NC(CC(=O)O)C(=O)NC(CC(=O)O)C(=O)NC(CCCCN)C(=O)O)NC(=O)C(CC(=O)O)N)O

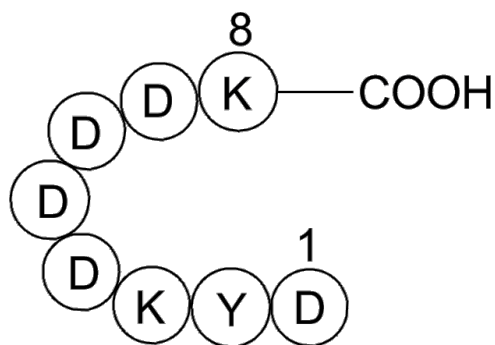
Formula $C_{41}H_{60}N_{10}O_{20}$ M.Wt 1012.97

Solubility ≥ 50.6 mg/mL in DMSO, ≥ 210.6 mg/mL in Water, ≥ 34.03 mg/mL in EtOH
Storage Store at -20°C , protect from light, stored under nitrogen

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

Purification of ribonucleoprotein (RNP) complexes composed of specific cellular rna by FLAG peptide coupled antisense oligonucleotide (ASO) pull-down [1]:

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Preparation of FLAG tag Peptide-Conjugated Antisense Oligonucleotide

1. Mix 50 μ L of 20 μ M (1,000 pmol/50 μ L) ice-cold oligonucleotide with 50 μ L of freshly prepared cold 1 M NaIO₄.
2. Incubate on ice for 10 min.
3. Add 1 mL of ice-cold 2 % LiClO₄/acetone and vortex.
4. Incubate on ice for 10 min.
5. Centrifuge the tube at 20,000 g at 4 °C for 10 min.
6. Carefully aspirate the supernatant to waste and add 1 mL of cold acetone.
7. Centrifuge the tube at 20,000 g at 4°C for 5 min.
8. During the centrifugation, prepare the FLAG-hydrazide solution.
9. Aspirate the supernatant to waste.
10. Dissolve the precipitated oligonucleotide in 12 μ L of 0.1 M sodium acetate (pH 5.2).
11. Add 12 μ L of 30 mM FLAG-peptide-hydrazide solution.

Preparation Method 12. Incubate the tube at room temperature for 30 min.

13. Add 10 μ L of 1 M NaCNBH₃.
14. Incubate the tube at room temperature for 30 min.
15. Add 60 μ L of ultrapure water and 10 μ L of 3 M sodium acetate (pH 5.2), then vortex.
16. Add 250 μ L of 100 % ethanol and vortex.
17. Incubate the tube at -80°C for 30 min.
18. Centrifuge the tube at 15,000 g for 10 min.
19. Carefully aspirate the supernatant to waste and add 1 mL of 80 % ethanol.
20. Centrifuge the tube at 15,000 g for 5 min.
21. Carefully aspirate the supernatant to waste. Repeat the wash step.
22. Dissolve the oligonucleotide pellet in 50 μ L of water.
23. Estimate the concentration of the FLAG-peptide-conjugated-oligonucleotide by measuring the absorbance at 260 nm and comparing with the absorbance of the original oligonucleotide solution.

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References:

[1]. Adachi S, Natsume T. Purification of noncoding RNA and bound proteins using FLAG peptide-conjugated antisense-oligonucleotides. *Methods Mol Biol.* 2015;1262:265-74. doi: 10.1007/978-1-4939-2253-6_16. PMID: 25555587.

Background

FLAG tag Peptide is an 8-peptide (Asp-Tyr-lys-Asp-Asp-Asp-ASP-ASP-Lys) containing an intestinal kinase restriction site. FLAG Tag peptide fusion system is used in immunoaffinity chromatography for protein identification and purification. FLAG tag Peptide label peptides allow elution under non-denatured conditions^[1,6]. The FLAG tag Peptide can be fused to either the N- or C-terminus of a given fusion protein.

The FLAG tag Peptide marker peptide fusion system comprises a unique and widely useful technique for protein identification and purification. When covalently attached to a solid support, the anti-Flag M1 antibody can be used for the rapid purification of FLAG tag Peptide fusion proteins in a mild, calcium-dependent affinity chromatography procedure. FLAG tag Peptide fusion proteins are typically purified to homogeneity in a single step, starting from a crude cell homogenate or supernatant, without ever exposing the protein to conditions other than physiological saline at pH 7.2 (with calcium or EDTA). The entire purification process can be carried out within several hours^[2-3].

The N-terminal FLAG tag Peptide fusion protein can be purified by agarose affinity gel ^[5]. A short-cut protein purification procedure, where mechanical cell disintegration and affinity purification by immobilization of the anti-Flag M1 antibody on magnetic glass beads are combined. The modified glass beads were added to yeast cells and mixed on a laboratory vortex mixer. The magnetic glass beads capture the target protein, while the yeast cells are disrupted. By making contact with a stationary magnet, the magnetic glass beads can very efficiently be separated from yeast cell debris. Chelating agents, such as EDTA, were capable of eluting the FLAG tag Peptide fusion proteins from the magnetic glass beads^[4].

References:

[1]. Einhauer A, Jungbauer A. The FLAG peptide, a versatile fusion tag for the purification of recombinant proteins. *J Biochem Biophys Methods.* 2001 Oct 30;49(1-3):455-65. doi: 10.1016/s0165-

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[2]. Chiang CM, Roeder RG. Expression and purification of general transcription factors by FLAG epitope-tagging and peptide elution. Pept Res. 1993 Mar-Apr;6(2):62-4. PMID: 7683509.

[3]. Prickett KS, Amberg DC, Hopp TP. A calcium-dependent antibody for identification and purification of recombinant proteins. Biotechniques. 1989 Jun;7(6):580-9. PMID: 2698650.

[4]. Schuster M, Wasserbauer E, Ortner C, Graumann K, Jungbauer A, Hammerschmid F, Werner G. Short cut of protein purification by integration of cell-disrupture and affinity extraction. Bioseparation. 2000;9(2):59-67. doi: 10.1023/a:1008135913202. PMID: 10892539.

[5]. Hopp TP, Prickett KS, Price VL, et al. A Short Polypeptide Marker Sequence Useful for Recombinant Protein Identification and Purification. Bio/technology (Nature Publishing Company). 1988;6:1204-1210. DOI: 10.1038/nbt1088-1204.

[6]. Li Y. Commonly used tag combinations for tandem affinity purification. Biotechnol Appl Biochem. 2010 Feb 15;55(2):73-83. doi: 10.1042/BA20090273. PMID: 20156193.

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