MDYKDHDGDYKDHDIDYKDDDDK；3xFLAG peptide

产品编号：MB2512
质量标准：>95%,BR
包装规格：10MG
产品形式：冻干粉

基本信息

分子式	C120H169N31O49S	结构式	MDYKDHDGDYKDHDIDYKDDDDK
分子量	2861.87		
CAS No.	-		
储存条件	-20℃，避光防潮密闭干燥		
溶解性 (25°C)	Water：2mg/ML ;TBS (5mg/ml);		
注意事项	溶解性是在室温下测定的，如果温度过低，可能会影响其溶解性。		
其他说明	为了您的安全和健康，请穿实验服并戴一次性手套操作。		

氨基酸序列：MDYKDHDGDYKDHDIDYKDDDDK(简称)
N-Met-Asp-Tyr-Lys-Asp-His-Asp-Gly-Asp-Tyr-Lys-Asp-His-Asp-Ile-Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys-C(全称)

技术指标

外观：..................冻干粉
纯度：..................>95% by LC-MS
溶解性：...............water 2mg/ML; TBS (5mg/ml);
储存条件：-20℃，避光防潮密闭干燥

相关产品推荐：

TBS 溶液经常用于配置 3xFLAG peptide 溶液。我司提供的 TBS 为 20X 浓缩液。使用前需要稀释。

MA0141	20 x TBS 浓缩液

用途及描述：科研试剂，广泛应用于分子生物学，药理学等科研方面，严禁用于人体。3X FLAG 肽是一种具有 3 次重复 DYKXXD 基序的合成肽。

使用方法推荐（来自公开文献，仅供参考）

一：常见储存液或者工作液配置方法
储存液配置：3xFLAG peptide 溶于 1X 的 TBS(pH7.4),浓度为 5mg/ml; 分装储存于-20℃，避免反复冻融。
工作液配置:3xFLAG peptide 常用工作液浓度为 100ug/ml ,可用于从 Anti-FLAG M2 亲和凝胶上洗脱 3xFLAG 融合蛋白。5 倍柱体积的工作溶液即可充分洗脱大部分 3xFLAG 融合蛋白

二：FLAG-Tag Protein Purification Protocol for Mammalian Cells

General Notes The following protocol is based on and optimized for over expressed FLAG-tagged proteins from mammalian cells (U2OS) grown in one 10 cm2 plate transfected at 90% confluence and harvested after 48 hours. This protocol works well for co-purification of interacting proteins (based on FLAG-Ring1B/Bmi1).

1 Re-suspend and Sonicate Cells Re-suspend the frozen mammalian cell pellet in 2 mL of FLAG-Purification Suspension Buffer (1XPBS, 1mM DTT, 1X Protease Inhibitor). This re-suspension

buffer does not have any lysis agents in order to preserve enzymatic activity. Purifications not harvesting for activity may use actual lysis buffersin addition to sonication (1% Triton X or other). This protocol uses sonication only.

Transfer the mixture to a clean 2 mL microtube and sonicate 2 times for 10 continuous seconds each, with 1 min pause in between using "Amplitude Setting 1 [out of 20]" on a Misonix XL- 2000 sonicator.

2 Prepare and Incubate Cell Lysate with Beads Centrifuge the cell lysate for 20 min at 12,000 g at 4o C to pellet lysate debris.

Use a 2 mL "micro" purification column and flush the column with 2mL of the FLAG-Purification Suspension Buffer.

Transfer 100 µL of M2 FLAG Affinity beads to the column and wash the gel away with 2 mL of FLAG-Purification Suspension Buffer.

Dry the nose (bottom) of the column and close it with the cap very tightly, then transfer the supernatants from the centrifuged cell lysate to the column gently avoiding the debris pellet.

Cap the column and incubate on a rotator at 4oC for 1.5 hours. This may be extended to overnight if enzymatic activity of protein is not a concern.

3 Wash The Column Wash the column after incubation with at least 10 mL of FLAG-Purification Suspension Buffer. If the column doesn' t flush by gravitational flow, centrifuge at low speed (~300-400 RPM) for just a few seconds.

4 Elution To lute the column by incubating the beads at 4 o C for 30 min with 100 µL of 3X FLAG Peptide at a 100 ng/mL working concentration [the "3X" in 3X FLAG Peptide is not any indication of concentration, it simply refers to 3 tandem repeats of FLAG sequence in the peptide]. You can make a 1 mL of the working concentration by mixing 20 µL of the commercially available 5 mg/mL stock with 980 µL of TBS.

At the end of the incubation centrifuge at low speed for a few seconds if column does not elute by gravitational flow. You may do additional elution steps to recover more protein.

三：Purification of Halo or FLAG tagged protein complexes from transiently transfected HEK293 cells for MudPIT mass spectrometry

1. Seed a 15 cm dish with ~2 x 107 cells. Allow cells to recover overnight.
2. Transfect with 7.5 µg of DNA expressing FLAG or Halo tagged protein of interest.
3. Harvest cells ~48 hours post transfection:
a. aspirate media,
b. wash with 10-20 ml ice-cold PBS,
c. add 10 ml ice-cold PBS and remove cells with a cell scraper and transfer cells to a 15 ml tube on ice,
d. spin at 2000 x g for 10 minutes at 4°C,
e. remove PBS; resuspend in ~1 ml ice-cold PBS and transfer to 1.5 ml microfuge tube.
f. spin at 2000 x g for 10 minutes at 4°C,
g. Freeze pellets in liquid nitrogen and transfer to -80°C (protocol can be stopped at this point).
4. Thaw cells on ice and resuspend in 300 µl mammalian lysis buffer containing protease inhibitors.
5. Pass through a 26G needle using a 1 ml syringe 5-10 times.
6. Spin at max speed 10 minutes at 4°C.
7. Transfer supernatant to a clean tube (protocol can be stopped at this point; freeze pellets in liquid nitrogen and transfer to -80°C).

8. Add 700 µl ice-cold PBS.
9. Spin at max speed 10 minutes at 4°C.
10. Transfer supernatant to a clean tube containing washed anti-FLAG agarose or HaloLINK resin. Leave final 10-20 µl to avoid disturbing pellet.
11. Incubate with mixing for 1 hour at 4°C.
12. Wash beads four times (spin at 2000 x g for 1 minute at 4°C, discard supernatant, add 1 ml ice-cold wash buffer and repeat).
13. Elute:
a. FLAG elution: Add 100 µl FLAG elution buffer and incubate 30 minutes at 4°C with shaking.
b. Halo elution: Add 100 µl Halo elution buffer and incubate 1-2 hours at 25°C with shaking.
14. Spin at 2000 x g for 1 minute. Transfer the 100 µl supernatants to an empty micro bio spin column (BioRad #732-6204) inserted into a 1.5 ml microfuge tube. Spin at 2000 x g for 1 minute.
15. Aliquot 20 µl of the eluate for analysis by SDS PAGE. Digest the remaining 80 µl eluate for analysis by MudPIT mass spectrometry.

Preparation of anti-FLAG agarose or HaloLINK resin

1. Vortex container of anti-FLAG agarose (sigma A220) or HaloLINK resin (Promega G1912) to resuspend.
2. Aliquot 100 µl slurry per purification into 1.5 ml microfuge tubes.
3. Wash 3 times with wash buffer (spin at 2000 x g for 1 minute at 4°C, remove supernatant, add 1 ml ice-cold wash buffer and repeat). Only remove final wash just prior to adding lysate.

【注意】

●我司产品为非无菌包装，若用于细胞培养，请提前做预处理，除去热原细菌，否则会导致染菌。
●部分产品我司仅能提供部分信息，我司不保证所提供信息的权威性，以上数据仅供参考交流研究之用。

活性化合物操作注意事项

1 **产品分装：**您收到货物后最好不要自己进行分包，因为分包环境、包装材料等因素可能导致分包后的产品变质；如您有特殊包装要求，请在订购时候与我们客服代表阐明，当然价格会做适当调整。对于开盖后，长期未使用的，请务必重新密封好，建议 Parafilm 封口膜，并按照相应储存条件使用。如果放置时间过长，超过产品有效期，建议您重新购买，以免影响实验质量。

2 储备液制备：大部分试剂的溶液形式稳定性较差，请优先采用现用现配的方式。 如需制备储存液，请选用合适溶剂，细胞培养类多选择 DMSO，储备液制备完成后请于零下 80 摄氏度储存，一般可以稳定存在 3-6 个月以上。在使用前，再对储备液进行稀释。避免储备液反复冻融。

3 细胞培养工作液制备：请根据个人需要正确计算浓度，稀释储备液或者直接用粉末配置工作液。由于大部分化合物是脂溶性的，所以使用水性溶剂（如 PBS）稀释时，可能会析出沉淀，可通过超声使固体重新溶解，不会对实验产生影响。如用 DMSO 作为溶剂，请确保 DMSO 最终使用浓度<0.3%,以避免细胞毒性。

灭菌方式，我们建议通过 0.22UM 微膜过滤方式除菌，请勿采用紫外，射线或者高温灭菌方式，否则会影响化合物活性，甚至破坏其结构导致彻底失活。

4 体内动物实验应用：由于很多化合物是脂溶性的，动物实验工作液配制失活，可能会需要加入一些药用辅料作为助溶剂，如吐温，CMC-NA，甘油等，具体需要客户查阅相关文献决定。如使用 DMSO，请确保 DMSO 的终浓度<5%,以避免毒性作用。给药剂量设计时候，可以参考下表

动物体表面积等效剂量换算表

物种	体重(KG)	体表面积(M2)	Km 系数
狒狒	12	0.6	20
狗	10	0.5	20
猴	3	0.24	12
兔	1.8	0.15	12
豚鼠	0.4	0.05	8
大鼠	0.15	0.025	6
仓鼠	0.08	0.02	5
小鼠	0.02	0.007	3

动物 A(mg/kg)=动物 B(mg/kg) X 动物 B 的 Km 系数/动物 A 的 Km 系数

5 关于产品到货处理及验收

您收到产品后，请及时查验产品的包装完整性，并对数量进行确认。对于很多微量的产品，数量低于 500MG 的，我们出厂前都是保证正确数量包装的。由于产品包装可能在运输过程中倒置，从而导致产品附着在管壁或者盖子上，这时候请不要先打开盖子，需正位放置轻轻拍打，使产品沉降到官底。对于液体产品，可以在 200 转左右稍作离心，官底收集液体，从而避免损失。

产品标签标示重量会有一定成了误差，在下面范围内均属于我司正常范围，望周知

标示重量范围	误差范围
1-20MG	0.1MG
50-500MG	1MG
>1G	3-5MG

为什么会看起来包装瓶是空的，如果您购买的产品的量非常小，同时有些产品在冻干的过程中粘附在管壁上形成薄薄的一层，可能观察不到产品的存在。您可以加入指定溶剂（参照操作手册）并涡旋或超声震荡使之完全溶解。

对于蜡状或油状的的产品很难取出称量它们的质量，我们建议您用合适的溶剂直接溶解该化合物；对于具有吸湿性的化合物，暴露在空气中会吸收水分，呈现液滴状，这种产品需要放置在干燥器中保存。