

BOT® Fluor 488 Conjugation Kit

Cat No: BIOK1974

DESCRIPTION

Immunofluorescence technique is to label a known antibody or antigen molecule with fluorescein. When it reacts with the corresponding antigen or antibody, certain amount of fluorescein is attached to the formed complex. Under the microscope, the fluorescent antigen-antibody binding site can be seen, and the antigen or antibody can be detected. The main features of this technology are strong specificity, high sensitivity, and fast speed.

Fluorescein-labeled antibody is abbreviated as fluorescent antibody (FA), which is currently widely used in immunopathology, cytochemistry, flow cytometry, virology, and autoantibody clinical immunodiagnosis. It's specific, sensitive, qualitative and localized immune Chemical reagents. The antibody used for labeling requires high specificity and high affinity. The antiserum used should not contain antibodies against normal tissues in the specimen. Generally, IgG and IgM need to be purified and extracted before labeling.

The fluorescein in this kit uses high-quality imported BOT®Fluor fluorescein. The principle of AF fluorescein-labeled antibody is to use the NHS group on BOT®Fluor and the free NH₂ on the antibody to chemically form an AF-Ab conjugate, namely Fluorescent antibodies. There are 86 lysine residues in an IgG molecule, and generally can bind 15-20 at most. An IgG molecule can bind 2- 8 molecules of BOT®Fluor fluorescein molecules. This kit can label 0.1-1 mg antibody.

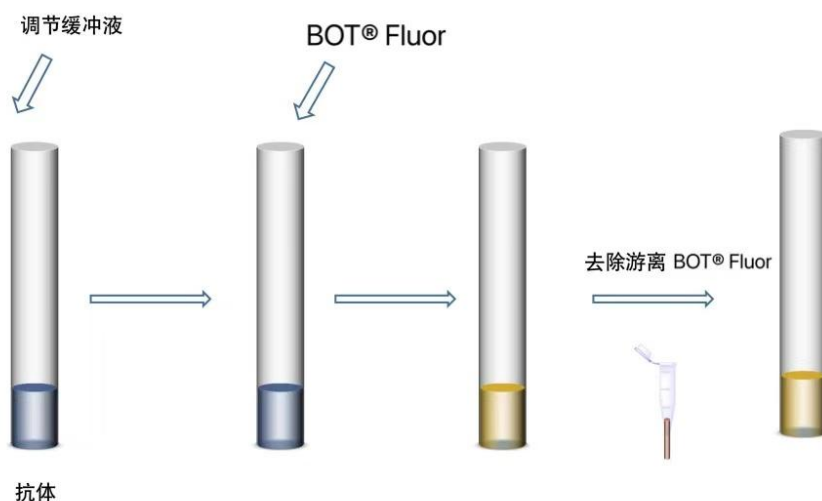
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PRODUCT INFORMATION

BOT® Fluor 488 荧光素	1 支 (避光)
调整缓冲液	50 ul
DMSO	150 ul
纯化柱	2 支
收集管	2 个



Steps

1. 抗体准备

- 1.1. 建议抗体浓度在 1-2mg/ml 之间。
- 1.2. 抗体中不要含有 BSA 或其它蛋白质成分。
- 1.3. 抗体缓冲液中不要含有氨基的盐(如: Tris, NaN₃ 等) , pH 在 6.5-8.5 为宜。

2. 抗体标记

- 2.1. 取出 BOT®Fluor 离心数秒, 将管中 BOT®Fluor 干粉甩至管底;
- 2.2. 管中加入 50 ul DMSO 用移液枪反复吹打或 vortex 混匀至 BOT®Fluor 完全溶

解;

2.3. 抗体中加入适量调整缓冲液 (每 100ul 抗体中加入 10ul 调整缓冲液);

2.4. 将溶解后的 BOT®Fluor 加入抗体中 (每 100ug 抗体中加入 4.0ul BOT®Fluor) ,
用移液枪反复吹打或 vortex 混匀。

2.5. 将抗体-BOT®Fluor 混合物置水平摇床或旋转混匀仪, 在摇动状态下室温避光(反应管可包裹锡箔纸)反应 1h。

注: 如果需要较高 F/P 值, 可适当延长抗体和 BOT®Fluor 偶联时间。

3.游离 BOT®Fluor 去除

3.1. 取出纯化柱, 将纯化柱 3000rpm 离心 2min。

3.2. 暂时保留收集管中的缓冲液。

3.3. 将纯化柱移至新的收集管上, 吸取抗体-BOT®Fluor 偶联物置纯化柱中填料的表面。如果样品体积小于 50ul, 应先用 3.2 保留的缓冲液将液体量补足 50ul。上样体积最大不要超过 100ul。

3.4. 待样品渗入填料后, 3000rpm 离心 2min。

3.5. 将抗体-BOT®Fluor 标记物 4°C 避光保存待用。

4.标记抗体保存

4.1. 标记抗体可 4°C 避光保存, 也可以根据需求选择加入 BSA、防腐剂、甘油等成分保证产品稳定。

4.2. 推荐保存液: 0.01M TBS(pH7.4)含 1% BSA, 0.03% Proclin300 和 50%甘油。

NOTES

1. Store the kit at 2-8°C, do not freeze.

2. The components of the kit may be upside down during transportation, causing liquid or dry powder reagents to stick to the tube wall or bottle cap. Please centrifuge before use to make the liquid or dry powder reagent attached to the tube wall or bottle cap settle to the bottom of the tube.
3. BOT®Fluor needs to be prepared for immediate use, and the dissolved BOT®Fluor cannot be stored for a long time.
4. The buffer in the purification column contains the toxic component sodium azide (NaN₃). Avoid contact with skin, eyes and mucous membranes when using it.
5. DMSO is slightly toxic, permeable to human skin and irritating to the eyes. Avoid contact with skin, eyes and mucous membranes when using it.
6. The dialysis, concentration and concentration determination of the antibody before labeling will all cause the loss of the amount of antibody. Therefore, when preparing the antibody before labeling, consider the most appropriate amount of antibody according to the specific situation.

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