iFluor 790 琥珀酰亚胺酯

产品编号：MB0290

质量标准：进分

包装规格：1MG

基本信息

分子量	1768.30
Excitation (nm)	787nm
Emission (nm)	812nm

简介：iFluor 染料是一系列荧光标记染料，可以覆盖整个可见光谱。所有 iFluor 染料都具有水溶性。它们的亲水性使有机溶剂的使用小化。iFluor 染料也具有比经典荧光标记染料更好的标记性能，如 FITC，TRITC，Texas Red ，Cy3 ，Cy5 和 Cy7。一些 iFluor 染料在某些抗体上明显优于 Alexa Fluor 标记染料。它们是用于标记蛋白质和核酸而不包含性能的便宜的荧光染料（替代 Alexa Fluor 染料）。每种 iFluor 染料的开发都与特定的 Alexa Fluor 或其他标记染料（如 DyLight 染料）的光谱特性相匹配。

琥珀酰亚胺基（NHS）酯被证明是用于胺修饰的试剂，因为形成的酰胺键基本上与天然肽键相同并且稳定。这些试剂通常是稳定的并且与脂族胺显示出良好的反应性和选择性。当琥珀酰亚胺酯化合物用于缀合反应时，需要考虑的因素很少：1）溶剂：在大多数情况下，活性染料应溶于无水二甲基甲酰胺（DMF）或二甲基亚砜（DMSO）中。 2）反应 pH：胺与琥珀酰亚胺酯的标记反应强烈依赖于 pH。胺反应性试剂与非质子化脂族胺基团反应，包括蛋白质的末端胺和赖氨酸的 β-氨基。因此，胺酰化反应通常在 pH 7.5 以上进行。通过琥珀酰亚胺酯进行的蛋白质修饰通常可以在 pH 8.5-9.5 下进行。 3）反应缓冲液：使用胺反应试剂时，必须避免使用含有游离胺（如 Tris 和甘氨酸）和硫醇化合物的缓冲液。广泛用于蛋白质沉淀的铵盐（例如硫酸铵和乙酸铵）也必须在进行染料缀合之前除去（例如通过透析）。 4）反应温度：大多数缀合在室温下进行。然而，特定标记反应可能需要升高或降低的温度。

Example protocol

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Protein stock solution (Solution A)

Mix 100 µL of a reaction buffer (e.g., 1 M sodium carbonate solution or 1 M phosphate buffer with pH ~9.0) with 900 µL of the target protein solution (e.g. antibody, protein concentration >2 mg/mL if possible) to give 1 mL protein labeling stock solution.
Note The pH of the protein solution (Solution A) should be 8.5 ± 0.5. If the pH of the protein solution is lower than 8.0, adjust the pH to the range of 8.0-9.0 using 1 M sodium bicarbonate solution or 1 M pH 9.0 phosphate buffer.
Note The protein should be dissolved in 1X phosphate buffered saline (PBS), pH 7.2-7.4. If the protein is dissolved in Tris or glycine buffer, it must be dialyzed against 1X PBS, pH 7.2-7.4, to remove free amines or ammonium salts (such as ammonium sulfate and ammonium acetate) that are widely used for protein precipitation.

Note Impure antibodies or antibodies stabilized with bovine serum albumin (BSA) or gelatin will not be labeled well. The presence of sodium azide or thimerosal might also interfere with the conjugation reaction. Sodium azide or thimerosal can be removed by dialysis or spin column for optimal labeling results.
Note The conjugation efficiency is significantly reduced if the protein concentration is less than 2 mg/mL. For optimal labeling efficiency the final protein concentration range of 2-10 mg/mL is recommended.

2. iFluor™ 790 SE stock solution (Solution B)

Add anhydrous DMSO into the vial of iFluor™ 790 SE to make a 10 mM stock solution. Mix well by pipetting or vortex.
Note Prepare the dye stock solution (Solution B) before starting the conjugation. Use promptly. Extended storage of the dye stock solution may reduce the dye activity. Solution B can be stored in freezer for two weeks when kept from light and moisture. Avoid freeze-thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

This labeling protocol was developed for the conjugate of Goat anti-mouse IgG with iFluor™ 790 SE. You might need further optimization for your particular proteins.
Note Each protein requires distinct dye/protein ratio, which also depends on the properties of dyes. Over labeling of a protein could detrimentally affects its binding affinity while the protein conjugates of low dye/protein ratio gives reduced sensitivity.

Run conjugation reaction

1．Use 10:1 molar ratio of Solution B (dye)/Solution A (protein) as the starting point: Add 5 µL of the dye stock solution (Solution B, assuming the dye stock solution is 10 mM) into the vial of the protein solution (95 µL of Solution A) with effective shaking. The concentration of the protein is ~0.05 mM assuming the protein concentration is 10 mg/mL and the molecular weight of the protein is ~200KD.
Note We recommend to use 10:1 molar ratio of Solution B (dye)/Solution A (protein). If it is too less or too high, determine the optimal dye/protein ratio at 5:1, 15:1 and 20:1 respectively.

2．Continue to rotate or shake the reaction mixture at room temperature for 30-60 minutes.

Purify the conjugation

The following protocol is an example of dye-protein conjugate purification by using a Sephadex G-25 column.

1. Prepare Sephadex G-25 column according to the manufacture instruction.
2. Load the reaction mixture (From "Run conjugation reaction") to the top of the Sephadex G-25 column.
3. Add PBS (pH 7.2-7.4) as soon as the sample runs just below the top resin surface.
4. Add more PBS (pH 7.2-7.4) to the desired sample to complete the column purification. Combine the fractions that contain the desired dye-protein conjugate.

Note For immediate use, the dye-protein conjugate need be diluted with staining buffer, and aliquoted for multiple uses.
Note For longer term storage, dye-protein conjugate solution need be concentrated or freeze dried.

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