

CypHer5E Mono NHS Ester

Product Specification Sheet

Introduction

Product codes

PA15401

PA15405

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

Store at 2-8°C in the dark. This product is light sensitive, protect from light. Do not use if the desiccant capsule in the pack is either pink or green.

Protect the CypHer5E Mono NHS Ester from light at all times. All exposures to light must be minimal and restricted to the time taken to complete a required operation.

We recommend that CypHer5E labelled antibodies are diluted to 0.5 mg/mL with PBS containing 0.1% BSA (w/v 1 mg/mL), remove any precipitate by centrifugation, dispensed into suitable aliquots and stored at -15°C to -30°C. Avoid repeated freeze-thaw cycles and protect from light.

Expiry

The expiry date is stated on the label and will normally be at least 16 weeks from the date of despatch.

Specifications

See batch analysis sheet enclosed with the product.

Description

CypHer5E is a red excited fluorescent, pH sensitive cyanine dye. It is non-fluorescent at basic pH and is fluorescent at acidic pH, with a pKa of 7.3. The CypHer5E Mono NHS Ester is suitable for labelling monoclonal antibodies and other proteins with CypHer5E.

CypHer5E anti VSV-G antibody has been used in G-protein coupled receptor studies using live cell lines. Agonist activation of G-protein coupled receptor results in the receptor being desensitized. Receptor desensitization leads to the internalization of the receptor from the neutral cell surface into acidic intracellular endosomes. A

large signal increase occurs when the CypHer5E anti VSV-G antibody is internalized alongside the receptor. In-house studies using anti VSV-G antibody labelled with CypHer5E have shown internalization in an agonist concentration- and time-dependent manner following binding to live cells.

Applications

This reagent has been shown to be suitable for live cell receptor internalization studies using the IN Cell Analysis system and confocal microscopes.

The success of the assay is dependent on a number of key steps being followed. We strongly recommend customers read the CypHer user manual (Product code 25-8010-12UM) before commencing any practical work.

The user manual can be found on our website at cytiva.com

Protocols

Described below is a general protocol for labelling a monoclonal antibody with CypHer5E Mono NHS Ester. The materials and procedures have been optimized for one particular preparation of monoclonal IgG antibodies. Other antibodies and proteins may also be labelled, however, the choice of buffers and labelling technique may need to be varied to produce optimal results.

Antibody purification

Step	Action
1	Antibody originating from mouse ascites fluid should be purified to a single immunoglobulin fraction by column chromatography, for example using a MAbTrap™ kit from Cytiva, which contains a HiTrap™ Protein G column. The manufacturer's instructions should be followed for the particular chromatography system chosen.
2	Scan all fractions for absorbance at 280 nm.
3	Collect all fractions containing the purified antibody and dialyse in PBS at room temperature for 4 hours with three changes of buffer or overnight with two changes of buffer.
4	Remove antibody from dialysis cassette and determine the antibody concentration (see step 1 in CypHer5E Mono NHS Ester conjugation to the antibody, on page 2).
5	It is recommended that the purified antibody is stored at 2-8°C for no longer than one week.

CypHer5E Mono NHS Ester conjugation to the antibody

Determination of antibody concentration

Step	Action
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1 Use the formula:

$$\frac{\text{Absorbance of antibody at 280 nm}}{\text{Molar Extinction coefficient of antibody}} = \text{Molar concentration of antibody}$$

UV absorbance at 280 nm (E280 0.1% = 1.4)

Molecular weight of IgG is assumed to be 150 000 Da

Molar Extinction coefficient (1.4 × 150 000) of antibody = 210 000 M⁻¹ cm⁻¹.

2 Proceed with the next part of the protocol.

Determination of CypHer5E Mono NHS Ester concentration

Step	Action
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1 Dissolve the whole vial of CypHer5E Mono NHS Ester dye in sterile, freshly opened DMSO, 100 µL for a 1 mg pack and 500 µL for a 5 mg pack.

2 Mix and sonicate for a few seconds to ensure the solution is homogeneous.

3 Add 5 µL of reconstituted dye to 4 mL of PBS/0.5 M Sodium Carbonate buffer pH 8.3 (9:1) in a glass vial and mix well.

4 Scan for absorbance from 240-750 nm with 0.125% DMSO as a blank. There should be one main peak at 500 nm, other minor peaks may be present.

5 Calculate the concentration of dye using the formula:

$$\frac{\text{Absorbance of dye at 500 nm} \times \text{dilution factor (800)}}{\text{Extinction coefficient of dye } (\epsilon)} = \text{Molar conc. of CypHer5E}$$

Assume ϵ CypHer5E Mono NHS Ester at 500 nm = 40 000 M⁻¹ cm⁻¹

6 Proceed with the next part of the protocol.

Conjugation of CypHer5E Mono NHS Ester to an antibody

Step	Action
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1 Dilute the antibody to 1 mg/mL in PBS and 0.5 M Sodium Carbonate buffer pH 8.3 (9:1); (v/v).

2 Calculate the amount of dye to be added to the 1 mg/mL antibody solution using the following equation. We recommend a 20 molar excess of CypHer5E Mono NHS Ester:
Molar concentration of antibody × volume of antibody solution × molar excess = Moles of CypHer5E required

3 Therefore the volume of dye to be added to the antibody solution can be calculated using the following equation:

$$\frac{\text{Moles of CypHer5E required}}{\text{Molar concentration of CypHer5E}} = \text{Volume of CypHer5E Mono NHS Ester}$$

2

Step	Action
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4 Add the required amount of CypHer5E Mono NHS Ester to the 1 mg/mL antibody solution and mix. Care must be taken to prevent foaming of the protein mixture.

5 Leave the solution to roll at room temperature in the dark for approximately an hour.

6 Separate labelled antibody from unconjugated dye using dialysis. We recommend using PBS overnight in the dark.

7 Remove the labelled protein solution from the dialysis tubing and dilute a sample so that the maximum absorbance is 0.5 to 1.5 AU at 280 nm and 500 nm using PBS/0.5 M Sodium Carbonate buffer pH 8.3 (9:1) This is for calculations of D:P only. For use in a CypHer assay dilute in PBS/BSA (see below).

8 Calculate the molar concentrations of dye and proteins as described in the *Estimation of final dye/protein (D/P) ratio* section below. The ratio of these values is the average number of dye molecules coupled to each protein molecule.

Estimation of final dye/protein (D/P) ratio

The calculation is corrected for the absorbance of the CypHer5E Mono NHS Ester at 280 nm. The factor of 0.16 accounts for this absorption. The estimated D/P ratio is calculated as follows:

$$\text{D:P} = \frac{A_{\text{DYE}} \times \epsilon_{\text{PROTEIN}}}{(A_{280} - 0.16 A_{\text{DYE}}) \epsilon_{\text{DYE}}}$$

A_{DYE} is the absorbance of the labelled antibody conjugate @ 500 nm at pH 8.3

A_{280} is the absorbance of the labelled antibody conjugate @ 280 nm at pH 8.3

$\epsilon_{\text{PROTEIN}}$ is the molar extinction coefficient of the antibody (210 000 M⁻¹ cm⁻¹ @ 280 nm)

ϵ_{DYE} is the extinction coefficient of the dye (40 000 M⁻¹ cm⁻¹ @ 500 nm).

The CypHer5E Anti VSV-G Antibody (PA45407) uses a D:P ratio of between 7.0-12.0 for optimum performance.

We recommend dilution to 0.5 mg/ml with PBS containing 0.1% BSA (see Handling Instructions). With PA45407 we recommend long-term storage at -15°C to -30°C, however, it can be stored at 4°C for up to 24 hours.

Please refer to the CypHer5E User Manual for more detailed information and protocols.

Related products

IN Cell Analysis System

IN Cell Analyzer 3000	25-8010-11
Granularity Analysis Module for IN Cell Analyzer 3000	63-0048-97
IN Cell Analyzer 1000	25-8010-26
Granularity Analysis Module for IN Cell Analyzer 1000	25-8010-30

CypHer

pCORON 1000 VSV-G Expression Vector	25-8008-51
pCORON 1000 SP VSV-G Tag Expression Vector	25-8009-92
CypHer5E Anti VSV-G Antibody (250 µg)	PA45407

Reference

1. Kreis, T.E., Embo J. 5, 931-941 (1986).

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