full spectrum cell analysis
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Reactive eFluor® Nanocrystals

Research Use Only

Introduction

Reactive eFluor® Nanocrystals are functionalized "reactive" nanocrystals that can be easily conjugated to proteins, antibodies, and other biomolecules of interest. The procedure below is designed to allow the user to employ eFluor™ Nanocrystals (NC) conjugates in a wide variety of fluorescence based assay, such as flow cytometric analysis, immunofluorescence, immunoassays and protein interaction assays. Please note that this protocol is presented as a "guide". The user may have to adjust the stoichiometry of the reaction components to optimize conjugation efficiency.

Useful websites

Wikipedia (http://en.wikipedia.org/wiki/EFluor_Nanocrystal)
General information regarding the eFluor nanocrystal properties can be found here.

Protocol A: eFluor® Nanocrystals (Amine) Conjugation Protocol

Materials

- 2nmol eFluor® Nanocrystals (Amine) (8-10 uM)
- 20X Borate Buffer (Pierce, cat. 28341): prepare 1X Borate Buffer in dH2O
- BS3, Bis (sulfosuccinimidyl) suberate (Pierce, cat. 21580)
- Prepare 40 mM BS3 solution in 50 mM Borate Buffer, pH8.5 (1X Borate Buffer), just before use.
- 6-20 nmoles of the biomolecule of interest (equivalent to 1-3 mgs of IgG)
  Note: biomolecule of interest must contain a primary amine group and be free of preservatives, such as BSA and azide. Biomolecule solution must be amine free (1X Borate buffer is recommended) and at high concentration for better coupling efficiency.
- Glass Vials
- PD-10 Desalting column (GE Healthcare, cat. 17-0851-01)
- Ultrafiltration units with 100 kDa molecular weight cutoff (Millipore, Amicon Ultra-15, cat. UFC910096)
  Note: If biomolecule is <60 kDa use ultrafiltration to separate un-reacted biomolecules from eFluor™ Nanocrystals (Amine) conjugates.
- Superdex 200 (GE Healthcare, cat. 17-1043-10 or cat. 17-5175-01)
  Note: If biomolecule is >60 kDa use HPLC or FPLC for optimal separation of un-reacted biomolecules from eFluor™ Nanocrystals (Amine) conjugates.
- Bulleted list of instruments here

Experimental Procedure

Step I: eFluor® Nanocrystals (Amine) Activation

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1. Transfer 2.0 nmoles of eFluor® Nanocrystals (Amine) to a glass vial and activate by adding 50uL of 40mM BS3 dissolved in 50mM Borate buffer (pH 8.5). Incubate the reaction mixture for 30 minutes at room temperature while gently mixing.
2. During the incubation period, equilibrate a PD-10 Desalting column with 1X Borate buffer according to manufacturer's instructions.
3. Load the reaction mixture to the PD-10 Desalting column and allow the sample to enter the gel bed completely. Elute the activated eFluor™ Nanocrystals (Amine) into a glass vial by adding 3-5 mls of 1X Borate buffer to the column.
   Note: Collect only the fluorescent colored fraction. To avoid sample contamination with excess cross-linker (BS3) do not collect any sample after the fluorescent colored fraction (use a black light to monitor elution).
4. Concentrate the purified activated eFluor® Nanocrystals (Amine) to 200ul using Ultrafiltration unit with 100 kDa molecular weight cutoff

Step II: Biomolecule Coupling

1. Add 6-20 nmoles of the biomolecule of interest to the purified activated eFluor® Nanocrystals (Amine).
   Note: The stoichiometry of the reaction must be optimized to ensure maximum coupling efficiency.
2. Incubate the reaction at room temperature for 1.25 hours under while gently mixing. Protect reaction from light.

Step III: Conjugate Purification

Biomolecules < 60kDa:

Add the conjugate to an Ultrafiltration unit with 100 kDa molecular weight cutoff and wash 5-6 times with 1X Borate buffer. Concentrate conjugate to <500µl.

Biomolecules >60kDa:

Purify the conjugate by FPLC or HPLC size exclusion chromatograpy using a Superdex 200 matrix. Pool the fluorescent fraction (use a black light to identify fluorescent fractions), and concentrate to < 500ul using an Ultrafiltration unit with 100 kDa molecular weight cutoff. Pool the fractions containing free biomolecule and quantify the amount of un-reacted material to determine the coupling efficiency

Protocol B: eFluor® Nanocrystals (Carboxyl) Conjugation Protocol

Materials

- 2nmol eFluor® Nanocrystals (Carboxyl) (8-10 uM)
- 20X Borate Buffer (Pierce, cat. 28341): prepare 1X Borate Buffer in dH2O (50 mM Borate, pH8.5)
- 50 mM Borate Buffer, pH7.2
- EDC (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) (Pierce, cat. 22980)
- Prepare 50 mM EDC solution in dH2O immediately before adding to eFluor® Nanocrystals (Carboxyl).

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Reactive eFluor® Nanocrystals

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- Biomolecule of interest
  Note: Biomolecule of interest must be free of preservatives, such as BSA and azide.
  Biomolecule solution must be amine free (50 mM Borate buffer, pH7.2 is recommended).
- Glass Vials
- Ultrafiltration units with 100 kDa molecular weight cutoff (Millipore, Amicon Ultra-15, cat. UFC910096)
  Note: If biomolecule is <60 kDa use ultrafiltration to separate un-reacted biomolecules from eFluor™ Nanocrystal (Carboxyl) conjugates.
- Superdex 200 (GE Healthcare, cat. 17-1043-10 or cat. 17-5175-01)
  Note: If biomolecule is >60 kDa use HPLC or FPLC for optimal separation of un-reacted biomolecules from eFluor™ Nanocrystal (Carboxyl) conjugates.

Experimental Procedure

Step I: eFluor® Nanocrystals (Carboxyl) Activation

1. Transfer 2.0 nmoles of eFluor® Nanocrystals (Carboxyl) to a glass vial and activate by adding 50 uL of 50mM EDC dissolved in dH2O. Mix well. There is no need to incubate at this point.
2. Add appropriate amount of biomolecule to the reaction mixture. Optimal coupling ratio may vary depending on biomolecule and must be determined by the end user.
3. Incubate the reaction mixture for 2 hours at room temperature while gently mixing

Step II: Conjugate Purification

Biomolecules < 60kDa:

Add the conjugate to an Ultrafiltration unit with 100 kDa molecular weight cutoff and wash 5-6 times with 1X Borate buffer (pH8.5). Concentrate conjugate to <500µl.

Biomolecules >60kDa:

Purify the conjugate by FPLC or HPLC size exclusion chromatograpy using a Superdex 200 matrix equilibrated with 1X Borate buffer (pH8.5). Pool the fluorescent fraction (use a black light to identify fluorescent fractions), and concentrate to < 500ul using an Ultrafiltration unit with 100 kDa molecular weight cutoff. Pool the fractions containing free biomolecule and quantify the amount of un-reacted material to determine the coupling efficiency.

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