
Cascade Blue Conjugation of Antibodies

Overview:

Cascade Blue is a UV-excitable dye that can be used for immunofluorescence labeling. When used with the 351/361 nm excitation lines of an Argon laser, it is not very bright; usually only extremely high density antigens can be well-resolved by Cascade Blue. However, when used with the 405 nm excitation line of a Krypton laser, it becomes a useful dye with a brightness approaching that of fluorescein. Emission is collected at 440 nm.

Refer to notes about the following procedures used by this protocol:

Column chromatography

Reagent storage

Contents:

Conjugation protocol

I. Preparation of antibody

II. Covalent conjugation

Materials, chemicals, and buffers

References

Conjugation protocol.

The entire conjugation can be performed in about a half-day. In addition to the materials listed below, you will need to have a solution of your antibody at a concentration (optimally) of at least 2 mg/ml. The extent of Cascade Blue conjugation to the antibody may depend on the concentration of antibody in solution; for consistent conjugations, use a consistent concentration. You should be familiar with how to use a desalting column and how to take absorbance spectra.

The reactive Cascade Blue molecule is unstable. Once the Cascade Blue is solubilized, it should be used immediately.

When first conjugating an antibody, a range of Cascade Blue to antibody concentrations should be compared. The protocol suggests 150 μg per mg of antibody; for a first-time titration of Cascade Blue, try a range of 40 to 600 μg Cascade Blue per mg of antibody (for instance, 40, 80, 160, 320, and 600 μg per mg). Compare each conjugate by staining (you should perform a titration of antibody on cells for each reagent to determine the optimal staining concentration). Select the conjugate with the brightest "positive" cells which still has low background on "negative" cells.

I. Preparation of antibody

Note: it is critical that sodium azide be completely removed from any antibody.

Dialyze or exchange over a column the antibody in "B Reaction Buffer".

Measure the antibody concentration after buffer equilibration. (For IgG, 1 mg/ml has an A(280) of 1.4). If the

antibody concentration is less than 1 mg/ml, the conjugation will probably be sub-optimal. If necessary, dilute the antibody to a concentration of 4 mg/ml.

II. Covalent conjugation

Cascade Blue is covalently coupled to primary amines (lysines) of the immunoglobulin.

Dissolve 5 mgs of Cascade Blue in 500 μ l anhydrous DMSO immediately before use. This is tedious and takes a bit of vortexing and time.

Add Cascade Blue to give a ratio of 150 μ g per mg of antibody; mix immediately. (See notes above about using different molar ratios of Cascade Blue to antibody).

Wrap the tube in foil; incubate and rotate at room temperature for 4 hours.

Remove the unreacted Cascade Blue and exchange the antibody into "Storage Buffer" by gel filtration or dialysis. The unreacted dye will have the apparent color on the column; usually, the antibody conjugate will be too low a concentration to be colored: do not make the mistake of collecting the antibody by color visualization! (You can use a hand-held UV lamp in a darkened room to visualize the conjugate--it will appear faintly blue in comparison to the buffer).

Materials, Chemicals, and Buffers

Materials:

For column separations, we often use one of two types of pre-poured columns:

For 1.25ml to 2.5ml sample volumes: PD-10 (Sephadex G-25M).

For <0.5 ml sample volumes: NAP5 columns (Sephadex G-25 DNA grade).

Chemicals:

Cascade Blue acetyl azide, trisodium salt, mw 607.42

DMSO - anhydrous dimethyl sulfoxide.

Note: keep the DMSO absolutely dry at all times. We keep the bottle in a dessicator. Pour out an amount of DMSO sufficient for your need and then pipette that; don't pipetter directly into the bottle.

NaHCO₃ - sodium bicarbonate, mw 84.01

NaCO₃ - sodium carbonate, mw 106

NaCl - Sodium Chloride, mw 58.44

TRIZMA pre-Set crystals 8.0 - Combination of Tris base and TrisHCl, average mw 141.8

NaN₃ – Sodium Azide, mw 65

Buffers:

"B Reaction Buffer"
100 mM carbonate, pH 8.4

To make 1 Liter:
8.4g NaHCO₃
pH to 8.4

Note: sodium azide cannot be added to this buffer

"Storage Buffer"
10 mM Tris, 150 mM NaCl, 0.1% NaN₃, pH 8.2

To make 1 Liter:
1.42g TRIZMA 8.0
8.77g NaCl
1g NaN₃
pH to 8.2

See hints on storing buffers.

References and credits:

This protocol is based on an original protocol devised by Michael Anderson.