Vybrant™ Cell Lineage Tracing Kit (V-22915)

Introduction

Molecular Probes’ Vybrant™ Cell Lineage Tracing Kit offers a convenient method for traditional lineage tracing (fate mapping) as well as cell–cell communication studies involving tissue recombinations. This kit is based on techniques that have proven successful for the study of neural tissue development in Xenopus embryos.1,2 With this method, an aldehyde fixable Cascade Blue labeled dextran is first injected into the desired parent cells. After differentiation, the low-level signal is amplified via an anti-Cascade Blue antibody and a secondary antibody conjugated to our excellent Alexa Fluor® 546 dye.

The dextran tracer conjugate can be fixed in situ using traditional formaldehyde fixation techniques and is compatible with standard paraffin-embedding techniques for tissue preparation. The Alexa Fluor 546 dye is spectrally similar to tetramethylrhodamine, yet brighter and more photostable. Moreover, the orange-red fluorescence of this dye is beyond the autofluorescence of many biomolecules, in particular, beyond that found in the yolk granules of Xenopus embryos and thus allows weak signals from well differentiated cells to be detected more reliably. In addition, the kit should also be useful in systems where high levels of endogenous biotin would render an avidin–biotin detection strategy difficult.

Materials

**Kit Contents**

- **Dextran, Cascade Blue anionic, lysine fixable** (MW = 10,000, Component A), three vials, each containing 0.5 mg of lyophilized powder
- **Anti-Cascade Blue, rabbit IgG fraction** (Component B), 30 µL of a ≥1 mg/mL solution in PBS, pH 7.2, 5 mM sodium azide
- **Alexa Fluor 546 goat anti-rabbit IgG (H+L) conjugate** (Component C), 30 µL of a 2 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, 5 mM sodium azide

Storage and Handling

Upon receipt, the kit components should be stored refrigerated at 4°C, protected from light. When stored properly, the kit components will remain stable for at least three months. For longer storage, the dextran conjugate (Component A) may be stored at -20°C, protected from light. Likewise, the antibodies (Components B and C) may be divided into small aliquots and stored at -20°C, protected from light. The frozen dextran and antibody aliquots are stable for at least six months. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Spectral Characteristics

The approximate absorption and emission peaks for the Alexa Fluor 546 dye are 556 nm and 573 nm, respectively. The dye can be viewed with filter sets appropriate for tetramethylrhodamine or Texas Red® dyes.

Experimental Protocol

The following protocol is written for use with Xenopus embryos; the method can be adapted for use with other model embryological systems and is compatible with whole-mount in situ hybridization.1-3 The fluorescent label is stable in Murray’s clearing solution (benzyl alcohol:benzyl benzoate, 1:2 (v:v)).

**Injection of Cascade Blue Dextran**

1.1 Dissolve the contents of one vial of Cascade Blue dextran (Component A) in 20 µL of sterile water. The final concentration will be 25 mg/mL.

1.2 Prepare a 100% MMR buffer consisting of 100 mM NaCl, 2 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES, pH 7.4.

1.3 For injection, transfer fertilized eggs/embryos to the injection dish containing 5% Ficoll 400 in 33% MMR buffer (prepared in step 1.2). This solution minimizes leakage form injected cells.

1.4 Inject fertilized eggs with 3–5 nL of Cascade Blue dextran solution, blastomeres at the 2- to 4-cell stage with 2 nL and blastomeres at the 6- to 8-cell stage with 0.25–1.0 nL.

1.5 Allow injected embryos to heal in the Ficoll/MMR solution to the early blastula stage, then transfer them to 10% MMR buffer (prepared in step 1.2), for further development.

**Detection of Cascade Blue Dextran**

2.1 Transfer injected embryos to a 3–5 mL glass vial and fix the embryos in 3.7% formaldehyde for 1–2 hours at room temperature. After fixation, rinse the embryos in phosphate-buffered...
saline (PBS) two times for 5 minutes each. At this stage, embryos can be saved for later use by transferring the specimens to 100% methanol and storing at -20°C. Embryos stored in methanol may be rehydrated by successive 5-minute washes in 75%, 50% and 25% methanol, and finally in PBS.

2.2 Wash the embryos in PBS containing 0.1% Triton® X-100 (PBST) three times for 5 minutes each. Washes are best performed in the vials completely filled with wash solution and rotated end-over-end.

2.3 Replace the wash solution with 10 µg/mL proteinase K (PK) in PBS and incubate the embryos for 15–20 minutes at room temperature. If significant damage occurs, decrease the incubation time. Permeabilization of the embryos with PK treatment is not absolutely required but it does increase the sensitivity of the detection. Do not perform the PK treatment if you plan to colocalize the lineage tracer with some other protein by immunostaining. If you elect not to perform the PK treatment, then proceed to step 2.6.

2.4 Remove the solution and wash the embryos in PBST twice for 5 minutes each.

2.5 Refix the embryos in 3.7% formaldehyde for 20–30 minutes at room temperature. Wash the embryos in PBST three times for 5 minutes each.

2.6 Block nonspecific protein binding sites by incubating the embryos in PBST containing 3 mg/mL BSA for 30 minutes at room temperature.

2.7 Dilute the anti-Cascade Blue rabbit IgG (Component B) 500-fold in fresh PBST/BSA blocking solution. Add 1 mL of the diluted antibody solution to the embryos and incubate with gentle agitation for 4 hours at room temperature or overnight at 4°C.

2.8 Remove the antibody solution and wash the embryos at room temperature in PBST five times, 1 hour each wash.

2.9 Dilute the Alexa Fluor 546 goat anti-rabbit IgG (Component C) 500-fold in fresh PBST/BSA blocking solution. Incubate with the embryos, protected from light, for 4 hours at room temperature or overnight at 4°C.

2.10 Remove the antibody solution and wash the embryos, protected from light, at room temperature in PBST five times, 1 hour each wash.

References

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