



Tech Tips

Xenographic Retention Chromatography (XRC)

Enzyme Labeling with ELF[®] 97 (Ex/Em 345/530nm)

Overview

This is a convenient procedure for preparing and outlining the steps for using ELF[®] 97 to detect recovered Cytokeratin 8/18 positive cells with the EpiSep[™] CK8/18 reagent kits. Recovered Cytokeratin 8/18 positive cells are labeled with a biotin-conjugated monoclonal antibody and require the use of a Streptavidin conjugated fluorophore for detection.

Note

This recommended procedure is intended to follow the "Labeling of Cytokeratin 8/18 Positive Epithelial Cells" procedure in the EpiSep[™] CK8/18 kits' directional inserts.

Materials & Reagents Required

Each sample requires 100 μ L Streptavidin-Alkaline Phosphatase Working Solution and 100 μ L of Fluorescent Substrate Working Solution. This procedure is for preparing 1mL of each solution.

- ELF[®] 97 Cytological Labeling Kit #2, with Streptavidin-Alkaline Phosphatase conjugate (Molecular Probes Cat. # E-6603)
- 2mL microcentrifuge tubes.
- 0.2 μ m pore-size filter.

Important

Note that all reagents referred to in this procedure are included in the ELF 97 Cytological Labeling Kit (Molecular Probes cat. # E-6603)

Preparation of Streptavidin Alkaline Phosphatase Working Solution (SA-WS)

- 1) Label a 2mL microcentrifuge tube SA-WS.
- 2) Add 980 μ L of Blocking Buffer (Component B)
- 3) Add 20 μ L Streptavidin Alkaline Phosphatase Conjugate (Component H)
- 4) Vortex briefly or invert 10 times.

Preparation of 1mL of 10x Substrate Additives (Sub. Add.)

- 1) Label a 2mL microcentrifuge tube Sub. Add. 10x stock and date.
- 2) Add 980 μ L of Developing Buffer (Component C).
- 3) Add 10 μ L of Substrate Additive 1 (Component E).
- 4) Add 10 μ L of Substrate Additive 2 (Component F).

- 5) Mix on Microvial Rotator for 5 or more minutes, or vortex.
- 6) Store at 2 - 8°C for up to 48 hours.

Preparation of Substrate Working Solution (Sub-WS)

- 1) Label a 2mL microcentrifuge tube Sub-WS.
- 2) Add 850 μ L of Developing Buffer (Component C).
- 3) Add 100 μ L of 10x Substrate Additive, prepared in the previous section.
- 4) Add 50 μ L of 20x Substrate Concentrate.
- 5) Vortex briefly, invert 10 times.
- 6) Filter the solution into another vial using a 0.2 μ m pore-size filter.

Immunofluorescence Labeling

- 1) Add 100 μ L of Streptavidin-Alkaline Phosphatase Working Solution, prepared earlier. Wait 15 minutes.
- 2) Add 100 μ L of Wash Buffer (Component A). Wait at least 5 minutes.
- 3) Add 50 μ L of Wash Buffer (Component A). Wait at least 1 minute.
- 4) Add 50 μ L of Wash Buffer (Component A). Wait at least 1 minute.
- 5) Add 100 μ L of Fluorescent Substrate Working Solution. Wait 4 minutes.
- 6) Add 100 μ L of Wash Buffer (Component A). Wait 1 minute.

Proceed with the Nuclear Detection procedure.

Refer to the EpiSep[™] CK8/18 kits' directional insert for this procedure.

Technical Assistance

For technical assistance, contact the WaveSense, Inc. Technical Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

Customer Service

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