



# EpiSep™ *TechTips*

## *Xenographic Retention Chromatography (XRC)*

### Labeling with NeutrAvidin™ Fluorescein Conjugated

TT10-106

#### Overview

This is a convenient procedure for preparing and outlining the steps for using NeutrAvidin™ Fluorescein Conjugated 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 kit directional inserts.

#### Materials & Reagents Required

- NeutrAvidin Fluorescein Conjugated kit (Pierce cat. # 31006)
- 500µL freezer microvials ((brown preferred).
- 2mL microcentrifuge tubes (brown preferred).

#### Important

All reagents referred to in this procedure are contained in the NeutrAvidin Fluorescein Conjugated kit (Pierce cat. # 31006).

#### Preparation of NeutrAvidin Stock Solution

- 1) Label 10 x 500µL vials FITC-Stock.
- 2) Add 1.12mL of distilled water to Pierce NeutrAvidin Fluorescein Conjugated in a 2mL microvial.
- 3) Replace cap, vortex vial briefly, and invert 20 times.
- 4) Transfer 100µL to each labeled vial.
- 5) Prepare Working Solution or store frozen at -20°C.

#### Preparation of NeutrAvidin FITC Working Solution

- 1) Label a 1.5mL brown freezer vial (FITC-WS).
- 2) Add 975µL of distilled water to the labeled vial.
- 3) Dispense 25µL of Pierce NeutrAvidin Fluorescein Conjugated Stock Solution (prepared from lyophilized material).
- 4) Replace cap, vortex briefly, and invert 20 times.
- 5) Store refrigerated (2 - 8°C) in light tight container.

#### Immunofluorescence Labeling

- 1) Add 100µL of FITC-WS, prepared earlier. Wait 15 minutes.
- 2) Add 100µL of Wash Solution (from EpiSep™ CK8/18 kit). Wait 5 minutes.
- 3) Add 50µL of Wash Solution (from EpiSep™ CK8/18 kit). Wait 1 minute.
- 4) Add another, 50µL of Wash Solution (from EpiSep™ CK8/18 kit). Wait 1 minute.

#### Proceed with the Nuclear Detection procedure.

Refer to the EpiSep™ CK8/18 with DAPI or EpiSep™ CK8/18 with DAPI and MCF-7 Cells directional insert for this procedure.

#### Technical Assistance

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

#### Customer Service

To place an order, contact the WaveSense, LLC Customer Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

Copyrights© WaveSense, LLC

Doc# TT10-106 rev B

NeutrAvidin is a trademark of Pierce Biotechnology, Inc.