Standard Operating Procedure
for
Immunostaining

1.0 PURPOSE

To visualize protein localization within cells and tissue, through the use of primary antibodies against protein and secondary antibodies attached enzyme or flurophore.

2.0 SCOPE (Should include which Cores this SOP applies to)

This procedure applies to all personnel who will test mice on the challenging beam test within the Stem Cells Engraftment and *in vivo* Analysis Core.

3.0 PROCEDURE

3.1 Materials

1.1 Detergent (Trypsin, tween)
1.2 Phosphate Buffered Saline pH 7.2
1.3 Blocking agent (BSA, Normal Serum etc)
1.4 Mounting Media

3.2 General Procedures

- Rehydrate tissue in PBS
- Permabilize membrane with .5% triton
- Antigen Revealing (Optional)
    - Heat slides in 10mM sodium citrate buffer, pH 6.0 at 95-100C for 20min
    - Remove from heat and let stand at room temperature in buffer for 20min
    - Rinse in PBS 1min
- Block for 1hour at RT with 1% BSA
- Incubate with Primary (use manufacturer's dilution) overnight in 4C
- Wash in PBS 15min at RT 3x
- Incubate with Secondary(use manufacturer's dilution) for 1 hour in RT
- Wash in PBS 15min at RT 3x
- Mount cover slips using mounting media

Created by Ewa Stachowiak, 15 December 2011