

Tcf4 (TCF7L2) Antibody Staining
Embryonic muscle cryosections
Kardon Lab April 2012 (Allyson Merrell)

Protocol:

Embed embryos in OCT and cryosection (8-12 um thick)

Day 1:

5 min 4% PFA/PBS

3x3 min PBS

1 hour block in 5% goat or donkey serum/PBS at room temperature

Overnight at 4⁰C in 1⁰ antibody

Day 2:

5x3 min PBS

2 hours 2⁰ antibody at room temperature

3x3 min PBS

1 hour in Strep at room temperature

3x3 min PBS

5 min 4% PFA/PBS

3x3 min PBS

5 min Hoechst

2x3 min PBS

Rinse H₂O and coverslip with fluoromount

Reagents:

Tcf-4 (TCF7L2) Antibodies:

Rabbit mAb (1:100=0.7ug/ml final concentration): Clone C48H11 Cell Signaling
Technology 2569

Mouse IgG2a (1:100=10ug/ml final concentration): Clone 6H5-3 Millipore 05-511
Rabbit antibody is preferred

Biotin conjugated goat α rabbit

Jackson ImmunoResearch 111-065-144, stock diluted 1:1 in glycerol

1:500 (1.2 μg/ml) in 5% goat serum in PBS

Strep: Streptavidin conjugated Dylight 594 or 488 (Jackson ImmunoResearch Dylight 594: 016-510-084,

Dylight 488: 016-480-084, stock diluted 1:1 in glycerol)

1:200 (4.5 μg/ml) in PBS

Hoechst (Invitrogen, H1399)

10ul in 250 ml PBS

Fluoromount-G (Southern Biotech #0100-01)

All PBS washes and Hoechst staining in 250 ml buckets

1⁰ and 2⁰ antibodies and block in small volume (200-500ul) on slide, laying flat in humidified darkened plastic tray