

Tcf4 (TCF7L2) Antibody Staining
Adult muscle cryosections
Kardon Lab April 2012 (Malea Murphy)

Protocol:

Flash freeze freshly isolated muscle placed on cork in isopentane (cooled in liquid Nitrogen)
Cryosection (8-12 um thick)

Day 1:

5 min. 4% PFA
2 min. PBS X2
10 min. Citrate Buffer
30 min. Antigen Retrieve (2100 Retriever) in Citrate Buffer
2 min. PBS X2
5 min. in 3% H₂O₂ in PBS
2 min. PBS X2
1hr TNB buffer @ RT
Overnight in Tcf4 Ab (1:100) in TNB @ 4°C.

Day 2:

5 min. PBS X5
2 hrs. 1st Secondary Ab (Biotin conjugated) in TNB.
2 min. PBS X2
3 hrs. Vectastain ABC Reagent (14 µl of A and 10 µl of B per ml of PBS)
2 min. PBS X2
10 min. TSA Fluorecein or Cy3 in Dilluent Buffer (1:50) (both in TSA box)
2 min. PBS X2
5 min. 4% PFA
2 min. PBS X2
Rinse in water and let slide air dry
coverslip with Fluoromount with added Hoechst (20ul of 10 mg/ml in 25 ml Fluormount)

Reagents:

Tcf-4 (TCF7L2) Antibodies:

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

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

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

Citrate Buffer: 1.8 mM Citric Acid and 8.2 mM Sodium Citrate in water

TNB buffer: 0.5% PerkinElmer Blocking Reagent Cat # FP1020 in 1X PBS (store at - 20°C)

Vectastain ABC kit Cat #: PK-6100

PerkinElmer Tyramide Signal Amplification (TSA) Plus

Fluorescein Cat # NEL741

Cyanine 3 Cat # NEL744

Hoechst (Invitrogen, H1399)

Fluoromount-G (Southern Biotech #0100-01)

2100 Retriever at Electron Microscopy Sciences

<http://www.emsdiasum.com/microscopy/products/histology/retriever.aspx>

All PBS washes and Hoechst staining in 250 ml buckets

1^o and 2^o antibodies and block in small volume (200-500ul) on slide, laying flat in humidified darkened plastic tray