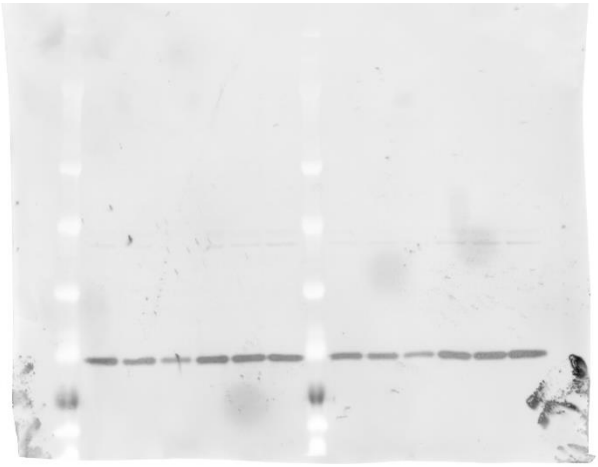
Technical Support Questionnaire – Western Blot

Name: TPI1 antibody
Catalog #: NBP1-31470
Lot Number: Batch number: 39624



Please upload an image of your western blot by clicking on the center of the box.

WB Image Description (Please provide labels for all lanes): L1: See Blue Plus 2 Marker L2-7: Pteropus alecto cell lysate. L8: See Blue Plus 2 Marker L9-14 Pteropus alecto cell lysate.

Sample Information:

Cell Line or Tissue: Immortalised Pteropus alecto kidney cell lysate (PaKiT03)
Species: Pteropus alecto
Treatment: Lanes 2-4 and lanes 9-11 were transfected with Poly I:C, an immunostimulant. Other lanes no treatment

Lysate Preparation:

Date of lysate preparation: 13/08/15
Lysis buffer used: 5% SDS
Reducing agent: DTT 0.5M
If boiled (temperature/time): 10 min

Controls:

Positive Control: none
Negative Control: none
Loading Control (please attach additional images if applicable): none

Protein Amount Loaded per lane: 10 ug

Antibody Storage Conditions: 4 degrees Celsius

Electrophoresis:

Gel Percentage: 4-12%
Electrophoresis Conditions: MOPS buffer from Life Technologies 1 mm
Voltage: 180V
Time: Until dye front reached bottom

Membrane Transfer:

Method (Submersion/Semi-dry): Submersion
Membrane Type (PVDF/Nitrocellulose): PVDF
Time: 60 min
Voltage: 170 milliamp

Blocking:

Blocking Solution: 5% Skim milk in tris buffered saline with 5% tween (TBST)
Time: overnight

Primary Antibody:

Dilution: 1/1000
Diluent Buffer: 5% skim milk in TBST
Incubation Time: 60 min
Incubation Temperature: room temperature

Washing Conditions:

Wash Solution: TBST
Time and Repetitions: 10 min, 3 times

Secondary Antibody

Manufacturer and Catalog #: Bio Rad #1706515
Secondary description: Goat Anti-Rabbit IgG (H + L)-HRP
Dilution: 1/2000
Diluent Buffer: 5% skim milk in TBST
Incubation Time: 60 min
Incubation Temperature: room temperature

Detection Method:

Detection: Chemiluminescence

Procedure: Following secondary incubation blots are washed twice in TBST for 10 min each and then washed once in tris buffered saline (TBS) for 10 min. Blots are then removed onto a glass plate and using Pierce ECL Plus Western Blotting Substrate is applied to the blot for 10 min. Blots are then blotted dried and left to dry before scanning on a GE Typhoon FLA 9000 on the fluorescence setting

Development Time: 10 min incubation with Pierce ECL Plus Western Blotting substrate

Molecular weight of band(s): ~28 kDa

Experimental Concerns and Observations: