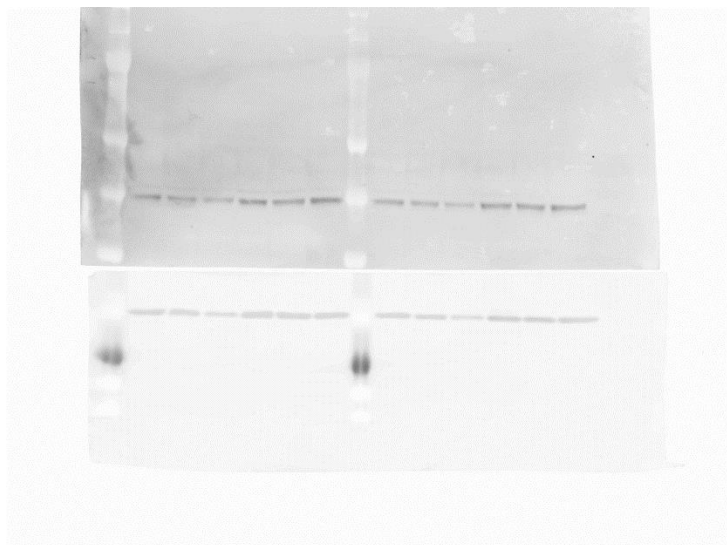


## **Technical Support Questionnaire – Western Blot**

**Name:** PGAM1 Antibody

**Catalog #:** NBP1-49532

**Lot Number:** Batch number: A2



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. (NOTE: membrane was cut in half and probed with separate antibodies. Top half was probed with beta-tubulin and bottom was probed with NOVUS PGAM1 antibody)

### **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): Top half of gel beta-tubulin

**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: 1/10000

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: 1/20000

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 at 350 PMT.

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



**Molecular weight of band(s):** ~28 kDa

**Experimental Concerns and Observations:** Probably could store this at 4 degrees. I have for a while and it still works fine.