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## NB300-817 Protocol

# Protocol specific for VAV3 Antibody (NB300-817)

Lysis:

Cell pellets were washed with ice-cold PBS. 1 ml of RIPA buffer was added per 1E8 cells and incubated on ice for 20 min, vortexing 2-3 times, briefly. The lysate was aliquotted into 1.5 ml microfuge tubes and centrifuged at 13,000 rpm for 5 min in a microfuge. The supernatant was transferred into clean tubes and its protein concentration was measured with BioRad protein assay. The concentration was then adjusted to 5 mg/ml with RIPA lysis buffer. An equal volume of 2 x SDS sample buffer was then added and the cell lysate was boiled for 5 minutes. Lysates were stored at -80C until use.(RIPA buffer = 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 5 ug/ml Aprotinin, 5 ug/ml Leupeptin, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS).

Tissue Lysis:

Tissue chunks were weighed and cut into approx 1mm cubes using a razor blade. The tissue was transferred to a handheld homogenizer and 3 ml of ice-cold RIPA buffer was added per 1g of tissue. The tissue was gently homogenised over 20 minutes on ice. The resulting lysate was aliquotted into 1.5 ml microfuge tubes and centrifuged at 13,000 rpm for 5 min in a microfuge. The supernatant was transferred into clean tubes and its protein concentration was measured with BioRad protein assay. The concentration was then adjusted to 5 mg/ml with RIPA lysis buffer. An equal volume of 2 x SDS sample buffer was added and the cell lysate was boiled for 5 minutes. Lysates were stored at -80C until use.(RIPA buffer = 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM PMSF, 1 mM EDTA, 5 ug/ml Aprotinin, 5 ug/ml Leupeptin, 1% Triton X-100, 1% Sodium deoxycholate, 0.1% SDS).

SDS PAGE:

Samples were run at 200V constant on a 12% acrylamide SDS-PAGE mini gel - using Biorad Mini-Protean 3 kit and protocols. Before loading samples had 5% (v/v) 2-ME added and were boiled for 3 minutes.

Transfer:

We used a Biorad Mini Trans-Blot, constant 100 V for 1 hour. Transfer Buffer was 20 mM Tris pH 8.0, 150 mM Glycine, 10% Methanol. We transferred to Millipore PVDF membrane and stained with Ponceau Red to evaluate the transfer.

Staining:

The membrane was blocked in 2.5% skimmed milk in TBS-T (TBS + 0.05% Tween-20) for 1 hr at room temperature with agitation. Primary antibody was incubated for 1 hr at room temperature with agitation. We used anti-goat-HRP Product at 1:3000 for 1 hr at room temperature with agitation. We washed with TBST three times after primary and secondary antibody, each wash lasting for 5-10 mins. ECL-plus (Amersham) was used rather than ECL, which is considerably more sensitive. Final detection was on autoradiography film.