

Specific applications

Direct detection of VSV-G-tagged proteins

Proteins containing the VSV-G epitope were separated electrophoretically, transferred to a PVDF membrane, detected directly with **Anti-VSV-G-POD** (P5D4), and visualized chemiluminiscently (Figure 3A.5). The proteins on the gel include:

- ▶ The calcineurin B GST fusion protein, tagged at the carboxy-terminus with the VSV-G epitope tag. Tagged protein (46 kD) was expressed by a plasmid in *Escherichia coli*. (*E. coli* cells containing tagged calcineurin B GST fusion protein were provided by Dr. Frank McKeon, Harvard Medical School.)
- ▶ Native VSV glycoprotein. The glycoprotein (66 kD) was expressed in mammalian (COS-1) cells by a full-length VSV-G cDNA that had been transiently transfected into the cells.
- ▶ The Na⁺/H⁺ exchanger-3 isoform (NHE3V) transmembrane protein, tagged with VSV-G epitope. Tagged protein (87 kD) was expressed in PS 120 cells (human fibroblast cell line) by a plasmid that had been stably transfected into the cells. (PS 120 cells containing tagged NHE3V protein were provided by Dr. Chung-Ming Tse, The Johns Hopkins University School of Medicine.)

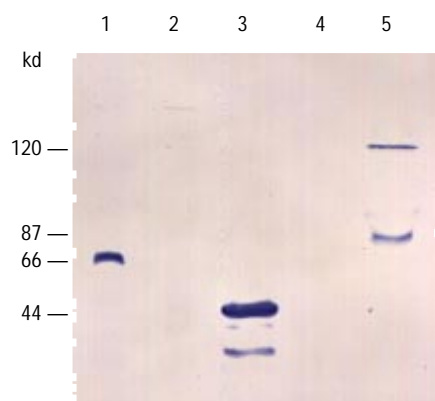


Figure 3A.5: Direct detection of VSV-G-tagged proteins on a Western blot with Anti-VSV-G-POD. Details of the Western blot and the source of the proteins are provided in the text. Lane 1: Lysate from transiently transfected COS-1 cells that express VSV glycoprotein (66 kD) from full-length VSV-G cDNA. Lane 2: Lysate from untransfected COS-1 cells; negative control for Lane 1. Lane 3: Lysate from *E. coli* cells that express VSV-G tagged calcineurin B GST fusion protein (46 kD); lower molecular weight bands represent breakdown products of the tagged protein. Lane 4: Lysate from *E. coli* cells that do not express the VSV-G tagged protein; negative control for Lane 3. Lane 5: Lysate from stably transfected PS 120 cells that express VSV-G tagged NHE3V protein (87 kD). The nonspecifically detected 120 kD band is also present in nontransfected PS 120 cells (C.-M. Tse, unpublished observation).

References

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