Neurokinin-1 (NK1) Human Receptor (Catalog #: RA25003) WB Protocol

1) Separate ~50 ug of whole cell or membrane protein on a 4-20% Tris-glycine gel.

2) Transfer the proteins from the gel onto a nitrocellulose membrane.

3) Incubate the nitrocellulose membrane in Blocking solution [5% non-fat dry milk / PBS] overnight at 4 degrees C.

4) Incubate the membrane with a 1:1,000 dilution of anti-NK-1 antibody (Novus Biologicals, Inc.,# NB300-119) in PBST-NFDM [PBS / 0.1% Tween-20 / 5% non-fat dry milk] for 2 hours at RT.

5) Wash the membrane with PBST-NFDM.

6) Incubate the membrane with HRP-conjugated goat anti-rabbit IgG diluted with PBST-NFDM for 1 hour at RT.

7) Detect antibody with an enhanced chemiluminescence reaction (ECL Western Blotting detection kit, Pharmacia).

FACS Protocol

1. Seed cells into small (T-25) flask. Add 5-10 ml media. Allow cells to grow to 80-100% confluence.

2. When flasks are confluent, empty media and add new media containing appropriate drug, hormone, or cytokine dose.

3. Allow flasks to incubate for 24 hours. After incubation period, remove 2 ml of media and place 1 ml into one of two
eppendorf tubes.

4. Empty off remaining media. Wash with 5 ml 1X PBS.

5. Add 2 ml PBS with azide (1% Goat serum). Scrape cells using a cell scraper.

6. Transfer cells to appropriate labeled FACSCAN tubes. Vortex all tubes

7. Incubate on ice for _ hour in the PBS with azide (1% Goat serum). Vortex all tubes after incubation.

8. Centrifuge at 1400 RPM for 5 minutes. Empty off media.

9. Resuspend in 1 ml of a 1:3,000 primary antibody solution (NB 300-119) [1 ul antibody per 3 ml PBS with azide (1%
Goat serum)].

10. Incubate on ice for 1 hour. Vortex all tubes after incubation.

11. Centrifuge at 1400 RPM for 5 minutes. Aspirate (or decant) supernatant.

12. Wash twice with 2 ml ice-cold PBS with azide (1% Goat serum).

13. Aspirate (or decant) supernatant and place tubes on ice.

14. Resuspend in 1 ml of a 1:500 secondary antibody solution [2 ul antibody per 1 ml PBS with azide (1% Goat serum)].

15. Vortex all tubes.

16. Cover with foil. Incubate for 30 minutes on ice.

17. Vortex after incubation. Centrifuge at 1400 RPM for 5 minutes.

18. Aspirate (or decant) supernatant. Wash twice with 2 ml ice-cold PBS with azide (1% Goat serum).

19. Aspirate (or decant) supernatant. Resuspend in 2% paraformaldehyde.

20. Read on flow.