



Neutralization Assay

ROCKLAND IMMUNOCHEMICALS produces antibodies to cytokines, interleukins and growth factors that are often used by investigators in biological assays, including neutralization assays to block bioactivity. Here we provide a generalized assay for the neutralization of a virus or cytokine. The information presented below is only intended as a guideline. The research should determine what protocol best meets their needs dependent on experimental goals. Remember to always follow safe laboratory procedures.

Materials Required

1. Neutralizing antibody. This may be purified antibody, ascites, antiserum or tissue culture supernatant (see Anti-Cytokines or Infectious Disease Antibodies for a listing of relevant primary antibodies) selection of anti Cytokine and infectious disease antibodies). Generally 5 mg/mL specific antibody is typically tested for neutralization.
2. Virus or cytokine to be neutralized. Virus is frequently measured in units between 1-100 of 50% tissue culture infectious dose (TCID50)
3. Target cells grown in 96 well tissue culture plates or other suitable assay format
4. Appropriate reagents to indicate target effect (see below) with appropriate platform for data collection

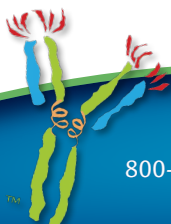
Procedure

1. Serial 1:1 or 1:2 dilutions of antibody, antiserum or tissue culture supernatant are made in a microtiter plate or other suitable format.
2. Target dose of cytokine is added to antibody and co-incubated for 1 hr at RT or 37° C. Include no antibody control, normal IgG (low EU Mouse IgG) or (low EU Rabbit IgG) control and no virus/cytokine control in defined wells.
3. Transfer co-incubated antibody - virus/cytokine to wells containing monolayers of target cells and allow incubation for 24 hr at 37° C.
4. Infectivity is identified by the presence of a cytopathic effect on target cells, or alternatively reduction of proliferative effects. Cytopathic effects can be assayed in varied ways. Plaque reduction neutralization testing (PRNT) has long been considered the standard for viral assays, but other assays can be used to measured cell viability or cell death such as MTT, MTS or Trypan Blue (see sample protocols below).
5. The median lethal dose (LD50) or neutralizing titer can be calculated by any suitable method. Neutralizing titer can be defined as the reciprocal of the highest dilution of serum or antibody at which: the target effect of the cytokine is neutralized in 50% of the wells or the infectivity of 100 TCID50 of the appropriate wild-type virus is neutralized in 50% of the wells (see Simmons et.al.)
6. Consult the available literature for the best system for your intended assay.

Cell Viability Protocols.

MTT - Thiazolyl Blue Tetrazolium Bromide (Sigma p/n M2128)

1. Make a 5 mg/ml MTT solution in sterile autoclaved PBS (5 mg/ml). Store at 4°C protected from light.
2. Add 25 µL MTT per well without removing medium from plate. Incubate 2 h at 37°C
3. Add 100 µL extraction buffer (12.5% SDS, 45% dimethylformamide, pH 4.7) and allow to incubate overnight at 37°C (to dissolve formazan-protein complexes in living/enzymatically active cells).
4. Read absorbance at 570nm. See Ross et.al. for details.





Amido Blue-black

1. Stain cells with 0.05% amido blue-black solution.
2. Fix cells with 10% formalin solution in acetic acid buffer.
3. Elute Amido blue-black stain in 0.15 mL of 0.05 M NaOH solution per well
4. Read ABS at 620 nm.

References

JE. Martin, et. al. West Nile Virus DNA Vaccine Induces Neutralizing Antibody in Healthy Adults during a Phase 1 Clinical Trial The Journal of Infectious Diseases (2007) 196:1732- 40.

T. Mosmann. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. (1983) Dec 16;65(1-2):55-63.

MH. Oliver, N K. Harrison, JE. Bishop, PJ Cole, GJ Laurent. A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors. (1989) J Cell Sci 92, 513-518.

M. Patricia D'Souza, Daniel la Livnat, James A. Bradac, Sandra H. Bridges. Evaluation of Monoclonal Antibodies to Human Immunodeficiency Virus Type 1 Primary Isolates by Neutralization Assays: Performance Criteria for Selecting Candidate Antibodies for Clinical Trials. J Infectious Diseases (1997);175:1056-1062.

C. Ross, M. B. Hansen, T. Schyberg & K. Berg. Autoantibodies to crude human leucocyte interferon (IFN), native human IFN, recombinant human IFN-alpha 2b and human IFN-gamma in healthy blood donors. Clin. exp. Immunol. (1990) 82, 57-62

CP Simmons, NL Bernasconi, AL Suguitan, K Mills, JM Ward, et al. Prophylactic and therapeutic efficacy of human monoclonal antibodies against H5N1 influenza (2007) PLoS Med 4(5): e178.

M. Wadhwa, A. Meager, P. Dilger, C. Bird, C. Dolman, R. G. Das, R. Thorpe. Neutralizing antibodies to granulocyte±macrophage colony-stimulating factor, interleukin-1a and interferon-a but not other cytokines in human immunoglobulin preparations. Immunology (2000) 99 113-123.

“Guidelines for Plaque Reduction Neutralization Testing of Human antibodies to Dengue virus” Initiative for Vaccine Research of the Department of Immunization, Vaccines and Biologicals. World Health Organization. Department of Immunization, Vaccines and Biologicals. http://whqlibdoc.who.int/hq/2007/WHO_IVB_07.07_eng.pdf

