

Protocol

Neutralization Assay

Rockland 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.

Reagents Required

Product	Preparation
Appropriate Reagents	For indicating target effect (see below) with appropriate platform for data collection.
Neutralizing Antibody	This may be purified antibody, ascites, antiserum or tissue culture supernatant. (See Anti-Cytokines or Infectious Disease Antibodies). Generally 5 mg/mL specific antibody is typically tested for neutralization.
Target Cells	Grown in 96-well tissue culture plates or other suitable assay format.
Virus/Cytokine (to be neutralized)	Virus is frequently measured in units between 1–100 of 50% tissue culture infectious dose (TCID ₅₀)

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 hour at room temperature 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 hours 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 measure 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:
 - a. the target effect of the cytokine is neutralized in 50% of the wells or
 - b. the infectivity of 100 (Median Tissue Culture Infectious Dose) TCID₅₀ of the appropriate wild-type virus is neutralized in 50% of the wells (see Simmons et.al.)

Procedure for Cell Viability: MTT - Thiazolyl Blue Tetrazolium Bromide

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 μ L MTT per well without removing medium from plate. Incubate 2 hours at 37°C.
3. Add 100 μ L 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 570 nm.

Procedure for Cell Viability: 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 *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 \pm 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.

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