



Neutralization Assay Protocol

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.

I. Reagents Required

Reagent	Preparation
Neutralizing Antibody	This may be purified antibody, ascites, antiserum, or tissue culture supernatant. 5 mg/mL specific antibody is typically tested for neutralization.
Virus/Cytokine (to be neutralized)	Virus is frequently measured in units between 1-100 of 50% tissue culture infectious dose (TCID50).
Target Cells	Grown in 96-well tissue culture plates or another suitable assay format.
Appropriate Reagents	For indicating target effect (see below) with appropriate platform for data collection.

II. 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. 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 TrypanBlue (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 the infectivity of 100 (Median Tissue Culture Infectious Dose) TCID50 of the appropriate.
 - b) the wild-type virus is neutralized in 50% of the wells.
6. Consult the available literature for the best system for your intended assay.

III. Cell Viability Protocols

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 µL MTT per well without removing medium from plate. Incubate 2 hours 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 570 nm.

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.