

Materials List for:

Rescue of Recombinant Newcastle Disease Virus from cDNA

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Materials

Name	Company	Catalog Number	Comments
DMEM	CORNING Cellgro	10-013-CV	Any supplier
OptiMEM	GIBCO	31985-070	
Lipofectamine 2000 (LPF2000)	Invitrogen	11668-019	
35% Bovine Albumin (BA)	Sigma	232-936-2	Any supplier
Trypsin-EDTA	CORNING Cellgro	25-052-CI	Any supplier
Penicillin/Streptomycin (PS) 100x	CORNING Cellgro	30-002-CI	Any supplier
Fetal Bovine Serum (FBS)	Hyclone	SH30070.03	Any supplier

Cell lines

A549 cells (catalogue number CRL-185), HEp-2 cells (catalogue number CRL-185), chicken embryo fibroblasts (catalogue number CRL-12203) and duck embryo fibroblasts (catalogue number CCL-141) are available from the American Type Culture Collection (ATCC, 10801 University Boulevard, Manassas, VA. 20110-2209 USA). All cell lines are maintained in a 37 °C incubator with 5% CO₂ in DMEM 10% FBS, 1% PS.

Embryonated chicken eggs

Embryonated chicken eggs can be obtained from Charles River Laboratories, Specific Pathogen Free Avian Supply (SPAFAS) Avian Products and Services (Franklin Commons, 106 Route 32, North Franklin, CT 06254, USA) and are maintained at 37 °C. Viability of the embryos is assessed with an egg candler. Eggs are infected when they reach 8-10 days old. Both infection and harvest of the allantoic fluid takes place under sterile conditions. All eggs are autoclaved and discarded following standard laboratory biosafety protocols.

Turkey red blood cells (RBC)

Turkey RBC can be purchased from Truslow Farms (201 Valley Road, Chestertown, Md 21620, USA) and stored at 4 °C. To prepare RBC for HA assay, wash 5 ml of the commercial stock with 45 ml of PBS 1x in a 50 ml conical tube. Centrifuge for 5 min at 1,000 rpm and carefully discard the supernatant. Dilute pelleted RBC 1:1,000 in PBS 1x for a final 0.5-1.0% concentration. Washed RBC can be stored at 4 °C for several days.

Plasmids

Plasmid preparations are obtained with any commercially available maxiprep kit following manufacturer's instructions, diluted in ddH₂O to a final concentration of 1 µg/µl and stored at -20 °C. DNA concentration and purity are assessed by spectrophotometry at 260 and 280 nm. Preparations with a 260/280 ratio higher than 1.8 are considered of acceptable quality for the rescue. Plasmid DNA quality is also routinely double-checked by agarose gel chromatography.

Viruses

The described protocol for rescue of the lentogenic NDV strain Hitchner B1 can be performed under biosafety level (BSL) 2 conditions. The Modified Vaccinia Ankara expressing the T7 RNA polymerase (MVA-T7) was described³⁴ and obtained from Dr. Bernard Moss. This virus is grown in confluent monolayers of chicken embryo fibroblasts and titrated in mammalian (A549 or HEp-2) cells. NDV stocks are grown in embryonated chicken eggs and titrated by IFA using polyclonal serum raised against purified virions. All contaminated material should be safely sterilized and disposed according to standard biosafety procedures.

Tissue culture media and solutions:

DMEM 10% FBS 1% PS: Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and Penicillin/Streptomycin (P/S): 445 ml of DMEM, 50 ml of heat-inactivated FBS, 5 ml of 100x commercial P/S solution. Store at 4 °C.

10x Phosphate buffered saline (PBS): 80 g of NaCl, 2 g of KCl, 11.5 g of Na₂HPO₄·7H₂O, 2 g of KH₂PO₄. Add ddH₂O up to 1 L. Adjust pH to 7.3. Sterilize by autoclave. Store at room temperature.

1x PBS: Dilute 10x PBS 1:10 with ddH₂O. Sterilize by autoclave and store at room temperature.

100x Ca/Mg : 1.327 g CaCl₂·2H₂O, 2.133 g MgCl₂·6H₂O and add ddH₂O up to 100 ml. Autoclave and store at room temperature.

1x PBS/BA/PS: 50 ml of 10x Phosphate buffered saline (PBS) in 437 ml ddH₂O. Autoclave and when cooled down to room temperature, add 5 ml 100x Penicillin/Streptomycin 3 ml 35% Bovine and 5 ml of 100x Ca/Mg. Store at 4 °C.