Materials List for:

Generation of Recombinant Influenza Virus from Plasmid DNA

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Materials

Name	Company	Catalog Number	Comments
DMEM	Invitrogen	11995-065	Store at 4°C
OptiMEM	Invitrogen	51985-034	Store at 4°C
Lipofectamine 2000 (LPF2000)	Invitrogen	11668-019	Store at 4°C
TPCK-trypsin	Sigma-Aldrich	T-8802	Store at -20°C
Bovine Albumin (BA)	Sigma-Aldrich	A7979	Store at 4°C
Trypsin-EDTA	Invitrogen	25300-054	Store at -20°C
Penicillin/Streptomycin (PS) 100X	Invitrogen	15140-122	Store at -20°C
Fetal Bovine Serum (FBS)	Hyclone	SH30070.03	Store at -20°C
V-bottom 96-weel plates	Nalge Nunc international	249570	

Embryonated chicken eggs

Embryonated 10-day-old chicken eggs can be obtained from Charles River Laboratories, Specific Pathogen Fee Avian Supply (SPAFAS) Avian Products and Services. Franklin Commons, 106 Route 32, North Franklin, CT 06254 USA. Eggs are incubated at 37°C preceding and after viral infection. Before and after viral infection, eggs are candled to determine viability of the embryos. It is very important to look for dead eggs before and after viral infection. Before infection a dead egg can be easily spotted by the absence of blood vessels as well as the absence of embryo mobility. When candled, live embryos move. After viral infection a dead egg (probably related to influenza virus infection) will be easily spotted by the bad appearance of the egg as seen by the smaller and bloody volume of allantoic fluid. Infected-eggs are discarded in double autoclavable bags and autoclaved following standard procedures.

Chicken red blood cells (RBC)

Chicken RBC can be purchased from Truslow Farms, 201 Valley Road, Chestertown, Md 21620. Store at 4°C. For HA assays, wash 5 ml of the chicken RBC with 45 ml of PBS 1X in a 50 ml centrifuge tube. Centrifuge for 5 minutes at 1000 rpms, RT. Discard carefully the supernatant and use a 1:1000 dilution of the pelleted RBC in PBS 1X (final concentration of 0.5-1.0% RBC).

Tissue culture supernatants and allantoic fluids

Both, tissue culture supernatants and allantoic fluids can be stored at 4°C for a short period of time. After confirming virus rescue, viruses from cell supernatants or allantoic fluid are stored at -80°C.

Plasmids

All plasmids are prepared using a plasmid maxi kit following manufacturer's recommendations. All plasmids are aliquot at concentrations of 1 µg/ml in ddH_2O and stored at -20°C. For short-term storage, the plasmid can be keep at 4°C. The concentration of the purified DNA plasmid is determined by spectrophotometry at 260 nm, with purity being estimated using the 260:280 nm ratio. Preparations with 1.8-2.0 260:280 nm ratios are considered appropriated for virus rescue purposes. Additionally, plasmid concentration and purity should be confirmed with agarose gel chromatography. Ambisense pDZ plasmids (6) containing the eight influenza A/PR/8/34 viral genes (7) are illustrated in Figure 2.

Viruses

The described protocol for rescuing influenza A/PR/8/34 can be performed under biosafety level (BSL) 2 conditions. Contaminated material, including tissue culture supernatants and embryonated eggs, should be sterilized before disposal. Rescue of other influenza virus may require higher BSL conditions and, therefore, special conditions/security measurements will need to be followed.

Tissue culture media and solutions

DMEM 10%FBS 1%PS: 445 ml Dulbecco's modified Eagle's medium (DMEM), 50 ml of Fetal Bovine Serum (FBS), and 5 ml of 100X Penicillin/Streptomycin (PS). Store at 4°C. This media will be used to maintain 293T and MDCK cells as well as for the transfections. DMEM 0.3%BA

1%PS: 495.7 ml of DMEM, 4.3 ml of 35% Bovine Albumin (BA). Store at 4°C. Just before use, add TPCK treated trypsin to a final concentration of 1 µg/ml. Infectious media.

10X Phosphate buffered saline (PBS): 80 g of NaCl, 2 g of KCl, 11.5 g of $Na_2HPO_4.7H_2O$, 2 g of KH_2PO_4. Add ddH_2O up to 1 liter. Adjust pH to 7.3. Sterilize by autoclave. Store at room temperature.

1X PBS: Dilute 10X PBS 1:10 with ddH_2O. Sterilize by autoclave and store at room temperature.