

Annex 5.1

Culture methods for Vero/hSLAM cells: maintenance, propagation, and preparation of frozen cell stock

NOTE: This document is intended to provide basic test method details and is not an SOP. Laboratories need to develop their own SOPs to suit their needs. The inclusion of reagents and products from specific manufacturers does not constitute an endorsement by the GMRLN or WHO.

- A. Maintenance of Vero/hSLAM cells
- B. Propagation of Vero/hSLAM cells
- C. Cryopreservation of Vero/hSLAM cells

A. Maintenance Vero-hSLAM cells

Vero/hSLAM cells being passaged for storage or for virus isolation should be manipulated in a Class-II Biological Safety Cabinet (BSC). Ideally, uninfected cells should be passaged in a BSC that is dedicated for clean cell culture. Only one cell line should be passaged at a time in the BSC. If a dedicated, clean BSC is not available, uninfected and infected cells must be passaged separately and the BSC must be decontaminated thoroughly between procedures. It is recommended to maintain separate stocks of cell culture medium for use for propagating infected and uninfected cells.

Reagents needed for maintenance of Vero/hSLAM cells

1. Dulbecco's Modified Eagle Medium (DMEM)
 - with 4,500 mg/L D-glucose (high glucose)
 - with L-glutamine
 - without sodium pyruvate
 - Alternate: EMEM
2. Antibiotics (100X)
 - 10,000 units/ml penicillin G and 10,000 µg/ml streptomycin sulfate in 0.85% saline

Add 5 ml of penicillin/streptomycin solution to 500 ml DMEM (label as DMEM-PS).
3. Trypsin-EDTA
 - 0.05% Trypsin (porcine pancreas), in 0.53 mM EDTA in HBSS without Ca⁺⁺ and Mg⁺⁺
4. Fetal Bovine Serum, Defined (FBS)

5. Geneticin® (G418), stock concentration 50 mg/ml, available as liquid. Final concentration is 400µg/ml (see note below).

Note: Geneticin Requirements of Vero/hSLAM cells

Because of the cost of Geneticin, several laboratories have investigated the stability of hSLAM expression during forward passages of the Vero/hSLAM cell line without the addition of Geneticin. The cells are fully susceptible to infection by wild-type measles viruses even after 15 passages without Geneticin in the medium. Therefore, Vero/hSLAM cells that are designated for virus isolation may be passaged forward 15 times in cell culture medium without the addition of Geneticin after recovery from liquid nitrogen stocks.

Network laboratories should only accept Vero/hSLAM cells from a WHO-approved source (RRL or GSL). Upon receipt, the cells should be passaged in medium containing 400µg/ml Geneticin. Laboratories should passage the cells 2 to 4 times in the presence of Geneticin to prepare a sufficient number of cells.

If preparing cells stocks for liquid nitrogen storage, add Geneticin® to the DMEM-PS to a final concentration of 400µg/ml. For 500 ml medium, add 4 ml of 50 mg/ml stock; label as DMEM-PSG).

B. Propagation of Vero/hSLAM cells

If expanding cells for frozen cell stock, add Geneticin® to the medium (final 400µg/ml).

When the cell layer is confluent, Vero/hSLAM cells can be passaged by trypsinization as with any other adherent cell line. Cells are usually maintained in T-25 or T-75 flasks, but the volumes given below can be adjusted for larger or smaller vessels.

1. If using T-25 or T-75 tissue culture flasks, wash cell monolayer once with 5 ml pre-warmed trypsin solution (or warm PBS) for about 30 sec to 1 minute to wash off the FBS.
2. Discard the wash medium into a beaker containing hypochlorite solution and add 5 ml pre-warmed trypsin solution to the cells.
3. Allow the flask to incubate on the bench top for 3-5 minutes. Remove most of the trypsin leaving just enough fluid in the flask to keep the monolayer wet (approximately 1 ml).
4. Place flask at 37° C for about 3-4 minutes. Observe flask every few minutes to avoid over-trypsinizing the cells. The cells should not dislodge until the flask is rapped firmly in the palm of the hand.
5. Gently mix with up and down with 1 or 2 ml pipette to break up any cell clumps. Resuspend cells in 5 ml DMEM-PS (or DMEM-PSG if preparing cell stock) plus 10% FBS and pipette up and down to break up clumps. Seed cells into flasks containing DMEM-PS (or DMEM-PSG) with 10% FBS. Split ratios of up to 1:5 are acceptable.

6. A 1:2 or 1:3 split usually will produce monolayers of sufficient density for virus isolation after 24 hours incubation (Total volume of medium required: T-25 flask is 10 ml/flask; T-75 flask is 30 ml/flask; T-150 is 50ml/flask).

Important notes:

Vero/hSLAM cells should be passaged at least once a week. Cells can be maintained, or growth slowed for several days by switching to medium containing 2% FBS.

If recovering cells for preparation of additional frozen cell stocks, Geneticin should be added to the medium.

Vero/hSLAM cells should be passaged forward no more than 15 times after recovery from liquid nitrogen when propagated for virus isolation.

C. Cryopreservation of Vero/hSLAM cells

It is important to prepare multiple vials of Vero/SLAM cells for frozen stock cells when cells have arrived and will be at the lowest passage level available. Cells can be frozen using any standard cryopreservation technique. Commercial freezing medium for cryopreservation can be purchased or the reagents and procedure described below may be utilised.

1. Vero/hSLAM cells should be passaged in medium with Geneticin (DMEM-PSG) to expand cells for a sufficient quantity of cells for multiple vials of cell stock (10-50 vials). This procedure provides volume measurements for a confluent T-150 flask of Vero/hSLAM cells, which is used to prepare 10 vials of cell stock.
2. Before beginning the procedure, label a sufficient number of screw-top cryovials with cell identity, passage number and date. Use pens or labels that can withstand liquid nitrogen. Prepare an ice bucket for use during the procedure.
3. Cells should be removed from the flask by trypsinization as described above (take care not to over-trypsinize). All of the cells from a T-150 flask should be placed in 10 ml DMEM-PS plus 10% FBS and pelleted by centrifugation at 1500 rpm for 10 min at 4°C. Discard supernatant.
4. Add 5 ml DMEM-PS (with antibiotics) containing 30% FBS to the cell pellet and resuspend the cells by gentle vortexing. Add an equal volume (5 ml) of DMEM-PS and 15% DMSO (reagent grade). Pipette gently up and down briefly to mix and dispense 1 ml into each of 10 plastic cryovials. Place immediately in ice.

It is important to work very quickly after addition of DMSO because prolonged exposure to DMSO at room temperature will be toxic to the cells. The vials should be cooled slowly using a programmed cell freezer or a commercial product designed for gradual temperature reduction (optimally -1°C /minute between 20°C and -70°C). Store vials in liquid nitrogen.

Perform a test recovery from the frozen stocks before discontinuing passage of the Vero/hSLAM cells (with Geneticin in the medium). Recover cells from one vial using the procedures described in step 5 below.

5. To recover cells from liquid nitrogen storage, remove the vial from the freezer and transport to the laboratory on dry ice. Thaw the cells quickly in a 37°C water bath and immediately place in a T-25 flask containing 10 ml DMEM-PS containing 10% FBS.

Allow the cells to attach to the flask for approximately 4 hours. After cells have attached, remove the medium with a pipet and replace with 10 ml of DMEM-PS with 10% FBS. Continue to observe cells daily. Expansion of adherent cells (monolayer) should be observable about 24 hours after recovery. It may take several days for the monolayer to become confluent. This is normal. If the cells do not recover properly (i.e. no or very few adherent cells and/or visible contamination), it will be necessary to prepare more stock of frozen cells from the original growing culture.