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DATA SHEET

Reagent:	CMV 759rD100-1 (GCV Resistant)
Catalog Number:	1912
Lot Number:	93141
Release Category:	A
Provided:	1 ml cell-free virus, $10^{4.75}$ $TCID_{50}$/ml (HFS cell assay).
Special Characteristics:	Ganciclovir-resistant mutant derived from CMV AD169 (Catalog #1910). Contains a 12 bp deletion in the UL97 region resulting in decreased ganciclovir phosphorylation. Also contains a Gly→Ala substitution at position 987 of the DNA polymerase conserved region V. The IC50 value of this lot to ganciclovir is 54 µM. Source of DNA: Human adenoidal tissues.
Recommended Storage:	Liquid nitrogen.
Contributor:	Dr. Karen Biron and Dr. Donald Coen.
References:	Sullivan V, Biron KK, Talarico C, Stanat SC, Davis M, Pozzi LM, Coen DM. A point mutation in the human cytomegalovirus DNA polymerase gene confers resistance to ganciclovir and phosphonylmethoxyalkyl derivatives. *Antimicrob Agents Chemother* **37**:19-25, 1993. Sullivan V, Talarico CL, Stanat SC, Davis M, Coen DM, Biron KK. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. *Nature* **358**:162-164, 1992.

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, AIDS Program, NIAID, NIH: CMV 759rD100-1 from Dr. Karen Biron and Dr. Donald Coen." Also include the reference cited above in any publications.

Propagation of Human CMV

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Introduction:

A variety of human diploid fibroblasts may be used for growing HCMV isolates. We use MRC-5, but other diploid human fibroblasts (ex: human foreskin fibroblasts; WI-38) may also be used. Experience will determine what the parameters are for each cell line (proper FBS concentration in the overlay, seeding density necessary for production of an actively growing, barely confluent monolayer optimal for over-seeding with virus, highest passage which will support good virus growth, etc.).

Most clinical CMV isolates do not produce good cell-free virus titers until after many passages in the laboratory, and even then, some never produce high titers. Low titer virus are, however, satisfactory for archiving plaque purified clinical isolate stocks. Recipients of these cell-free virus stocks can grow them for their own use, and revert back to cell-associated viral stocks.

CMV Stocks:

CMV clinical isolates C9208-5 (Cat. #2084), C9209-1 (Cat. #2082), and C9207-3 (Cat. #1909) are available as cell-associated virus in human foreskin fibroblasts. CMV laboratory isolates XbaF 4-3-1 (Cat. #1911) and 759rD100 (Cat. #1912) are available as cell-free supernatant obtained from infected human foreskin fibroblasts.

Culture Medium:

MEM (1X) with Earle's salts and L-glutamine (Gibco Cat. #11095-049). Supplement with 100 U/ml penicillin, 100 mg/ml streptomycin, and additional L-glutamine (1% final concentration). Fetal bovine serum should be added as described below.

I. Propagation of cell-free virus:

1. If primary human foreskin fibroblasts are used, they should be from passage 10-14 (P10-14). MRC-5 or WI-38 cells are available from the ATCC at P13-P18, and should be used before P25. MRC-5 cells obtained from BioWhittaker may be used through P30. When frozen cell stocks are used, they should be plated out without centrifugation, and residual DMSO removed by changing the culture medium as soon as the cells have attached.

2. Grow the host cells to near confluency in T-150 flasks (cultures should be actively growing). To infect, pour off all but approximately 5 ml of medium and infect the monolayer with CMV at an MOI of 0.01 to 0.05.

3. Incubate the culture for 90 minutes at 37°C, then add 40-50 ml of culture medium containing 4% FBS.

4. Maintain the cultures in medium with 4% FBS until the infection is well established and CPE is strongly evident. CPE should be at a +3 to +4 level (up to 90% or more of total cells infected, with dense clumps of balloon cells present). At this point, remove the culture medium and replace it with medium containing 2% FBS.

5. When the monolayer just begins to take on a lacy appearance, reduce the volume of medium to about 10 ml per flask. Continue to culture the cells,

changing the medium if necessary.

6. After approximately 7 days, begin collecting and pooling the culture supernatant, replacing the volume collected with 10 ml fresh culture medium each time. Collect supernatant daily until the monolayer has almost disappeared. We obtain the highest titers (10^7 pfu.ml) from 10-12 day cultures, or when the monolayer has an open lacy appearance and the cells are detaching. The timing could be different in other cell types, and the process will be faster if a higher MOI is used.

7. *Immediately* after each day's collection, sonicate the pooled supernatant using a water bath model sonicator for about 30 seconds to 1 minute to release virus from cell debris. Centrifuge the supernatant at low speed (450 RCF of approximately 1800 RPM) to eliminate debris.

8. Store the clarified supernatant at room temperature (NOT refrigerated) for up to three days to preserve titer. After several days of collection, pool the supernatants.

9. For long term storage, repeat sonification of the clarified virus, vortex, aliquot, and store aliquots in vapor phase liquid nitrogen. There is no need to freeze the stocks slowly. Stocks are most stable at temperatures < -125°C.

II. Propagation of cell-associated virus:

1. Prepare host monolayer cells as described in steps 1 and 2 above.

2. Thaw the frozen cell-associated CMV stocks rapidly in a 37°C water bath and co-seed or top-seed the entire contents of the thawed vial onto the established monolayers. Allow the cells to attach (this takes several hours).

3. Once the cells have attached, remove the culture medium and replace it with fresh culture medium containing 6-8 % FBS. Monitor the infection over the next several days, changing the medium as necessary.

4. If the virus does not appear to be spreading, trypsinize the cultures and redistribute them into new flasks without adding additional cells. This process breaks up the infected foci and allows better spreading.

5. Determine the titer of cell-associated virus based on a visual estimation of the percentage of viable cells in the growing monolayer (good estimates are based on personal experience with the cultures).

6. When infection is adequate, trypsinize the cultures and freeze the cells in aliquots containing at least 10^5 viable infected cells per ml. The freeze medium should contain 10% DMSO and 20% FBS. Freeze the cells slowly using a programmable freezer, and store the aliquots in liquid nitrogen or a -130°C freezer.

Last Updated: December 17, 2018