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DATA SHEET

Reagent:	CMV GDGrP53 (Ganciclovir Resistant)
Catalog Number:	1670
Lot Number:	3 96005
Release Category:	C
Provided:	1 ml cell-free virus, 2.8 X 10^4 PFU/ml.
Special Characteristics:	Polymerase ganciclovir-resistant mutant of human cytomegalovirus. Isolated by transferring the ganciclovir resistance mutation contained in the pol gene of mutant 759^{r}Dl00 into wild type strain AD169. CMV GDG^{r}P53 is cross-resistant to HPMPA and HPMPC and DHPC, and to ganciclovir phosphate. Host Site: Primary human foreskin fibroblasts. Also infects other human cells. Sterility: Negative for bacteria, fungi, and mycoplasma. Preparation: Propagate in human foreskin fibroblasts maintained in DMEM supplemented with 10% fetal bovine serum, 0.03% L-glutamine, and $NaHCO_3$ buffer (DME-10). Infect cells at an MOI of 0.01–0.1 pfu/cell (dilute stocks as necessary in DME-10) using volumes that just cover the monolayer (e.g., 1 ml per 100 mm dish). Incubate at 37°C for 1 hour, gently shaking the dish every 15 minutes to ensure even coverage. Remove the inoculum and maintain the cells in fresh, uninfected DME-10 until generalized cytopathic effects appear. Infection can be accelerated by trypsinizing and replating the cells. To harvest virus, scrape the cells into the medium, sonicate for 45 seconds using a horn cup sonicator at full power, and clarify the supernatant by centrifugation. Add glycerol to 10%, and freeze aliquots in liquid nitrogen. Original Source: Derived by marker transfer from mutant 759^{r}D100 and wild type strain AD169.
Recommended Storage:	Liquid nitrogen.
Contributor:	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.

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, AIDS Program, NIAID, NIH:GDGrP53 from Dr. Donald Coen." Also include the reference cited above in any publications.

Corporate requests should be directed to Dr. Donald Coen, Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, 250 Longwood Ave., Boston, MA 02115.

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