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DATA SHEET

Reagent:	GHOST (3) CXCR4+ Cells
Catalog Number:	3685
Lot Number:	032599
Release Category:	C
Provided:	1 vial frozen cells.
Cell Type:	Derived from HOS cells. Stably transduced with MV7neo-T4 retroviral vector, and stably cotransfected with the HIV-2 LTR driving hGFP construct and the CMV IE driving hygro-resistance construct.
Propagation Medium:	High glucose DMEM, 90%; fetal bovine serum, 10%. Supplement with 500 µg/ml G418, 100 µg/ml hygromycin, pen/strep, and 1 µg/ml puromycin **[NOTE: GHOST (3) Parental Cell Line is puromycin sensitive, do not supplement with puromycin.]**
Freeze Medium:	FBS, 90%; DMSO, 10%.
Sterility:	Negative for mycoplasma, bacteria, and fungi.
Special Characteristics:	GHOST (3) Parent Cell Line: Progenitor cell line used to develop GHOST (3) indicator panel. This cell line can also be used to introduce new HIV/SIV coreceptors to create novel indicator lines for infection analyses. Stable transduction of new genes can be achieved using puromycin resistance gene encoding vectors (e.g., pBABE-puro). They are adherent cells. GHOST Cell Transformants: Indicator cells for HIV-1, HIV-2, or SIV infection with uncloned, primary isolates, molecular clones, or pseudotyped virus. The puromycin-resistant cells are pools rather than clones for human coreceptor expression. The cells can be used to titer virus, evaluate drug sensitivities, or phenotype coreceptor use in conjunction with other GHOST cell lines. Infection is detected via induction of the hGFP gene as little as 24 hrs post-infection. Adherent cells. NEGSY timecourse on GHOST3 cells

NFNSX timecourse on GHOST3 cells

Recommended Storage: Liquid nitrogen

Contributor: Dr. Vineet N. KewalRamani and Dr. Dan R. Littman.

References:

Morner A, Bjorndal A, KewalRamani V, Littman DR, Inoue R, Thorstensson R, Fenyo EM, Bjorling E. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* **73**:2343-2349, 1999.

NOTE:

Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: (specify reagent) from Dr. Vineet N. KewalRamani and Dr. Dan R. Littman." Also include the reference cited above in any publications.

Patent pending. Scientists at for-profit institutions or who intend commercial use of this reagent must contact the New York University Office of Industrial Liaison at the following email address: abram.goldfinger@nyumc.org

Cell Line (Single Receptors)	Cat. No.	Lot No.	Cell Count	Comments
GHOST (3) Parental Cell Line	3679	5 042038	2.5 x 10^6/ml	Viability, 94%.
GHOST (3) CCR1	3680	3 030506	3.5 x 10^6/ml	CCR1 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) CCR2b	3681	3 020010	1.3 x 10^6/ml	CCR2b gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) CCR3	3682	2 011254	5.6 x 10^6/ml	CCR3 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) CCR4	3683	3 040919	6 x 10^6/ml	CCR4 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) CXCR4	3685	5 032599	1.5 x 10^6/ml	CXCR4 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) BOB/GPR15	3686	3 020011	2 x 10^6/ml	BOB/GPR15 gene introduced via retroviral infection withMLV BABE-puro vector.
GHOST (3) Bonzo/STRL33	3687	3 031152	4.8 x 10^6/ml	Bonzo/STRL33 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) V28/CX3CR1	3939	1 98001	2 x 10^6/ml	V28/CX3CR1 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) CCR8	3940	3 040128	1.3 x 10^6/ml	CCR8 gene introduced via retroviral infection with MLV BABE-puro vector.
GHOST (3) Hi-5	3944	5 042039	2.3 x 10^6/ml	GHOST (3) CCR5 cells additionally transduced with the non-selectable MX-CCR5 retroviral vector and sorted for high CCR5 expression. Cell surface CCR5 expression is higher than GHOST (3) CCR5 cells. Developed as control cells for comparison to infections of GHOST (3) X4/R5, GHOST (3) R3/X4/R5, and other single coreceptorcell lines.
Cell Line (Multiple Receptors)				

GHOST (3) X4/R5	3942	4 523472	1.2 x 10^6/ml	GHOST (3) Hi-5 cells additionally transduced with the non-selectable MX-CXCR4 retroviral vector and sorted for high CXCR4 expression. Useful for quickly assessing relative infectivity of virus with unknown coreceptor tropism, and for neutralization sensitivity studies of polytropic viruses.
GHOST (3) R3/X4/R5	3943	3 032593	3 X 10^6/ml	GHOST (3) CCR3 cells additionally transduced with the non-selectable MX-CCR5 and MX-CXCR4 retroviral vectors and sorted for high CCR5 and CXCR4 expression. Useful for quickly assessing relative infectivity of virus with unknown coreceptor tropism, and for neutralization sensitivity studies of polytropic viruses.

Research Chart:

Care and use of GHOST (3) HIV indicator cells (18feb98)

Please expand and freeze down early (no more than 10-15) passages of these cells as their phenotype changes over passaging.

Maintenance medium:

- High glucose DMEM + 10% FCS (any cheap brand) + pen/strep
- 500 µg/ml G418
- 100 µg/ml Hygro (reduce to 50 µg/ml if cells appear too sensitive) & for all coreceptor encoding cells (i.e. NOT PARENTAL) add
- 1 µg/ml puromycin

Note to previous users of GHOST (34) cells: GHOST (3) cells express a uniformly high level of human CD4 and are significantly more sensitive than the analogous (34) cells in infection assays. However, one disadvantage of the (3) line is that they express a detectable, albeit weak, level of endogenous CXCR4 on their cell surface. This corresponds to an increased susceptibility to CXCR4-tropic virus infection on all GHOST (3) cell lines. Nonetheless, the GHOST (3) cells transduced with exogenous CXCR4 are 10-20 fold more sensitive in infection assays with CXCR4-tropic virus than the other GHOST (3) pools.

Typical infection protocol for GFP analysis

All HIV/SIV handling should adhere to standard BL-3 protocols

Day 0, Infection preparation:

- Seed 2.5 x 10^4 cells per well of a 12 well plate the day before infection
- Cells can be plated in nonselective medium for single round infection exps

Day 1, Virus infection:

- Apply virus in the presence of 20 µg/ml polybrene to enhance infection efficiency. Cells are sensitive to DEAE/Dextran.
- Preferably, infections should be performed in a total volume of 300 µl per well of a 12-well plate. After 2 hr incubation in a 37°C humidified CO2 chamber, virus and polybrene should be replaced with 1 ml media.
- Alternatively, infections can be performed in 0.5 ml total volume overnight. Replace virus-containing medium the next day.

48 hr post-infection, Harvest and Analysis:

- A sample infection time course is attached. hGFP fluorescence indicating positive infection is depicted along the abscissa (FL1-H). Cells can be analyzed as little as 24 hr after infection; however the mean GFP fluorescence of the positively infected cells will be only 10-fold greater than mock infected cells. 48 hr post-infection, the mean GFP fluorescence intensity is greater than 20-fold over background. At the same time there is no discernable difference in the percentage of positively infected cells between 24 and 48 hr suggesting that the GFP read-out reflects a single-round infection dynamic. At 72 hr post-infection, the increase in mean GFP fluorescence of the infected cells is minimal compared to 48 hr.

- However, with a replication competent virus stock, two problems may arise. If the stock applied is of high titer, considerable cell death will be apparent beyond 48 hrs reducing the number of detectable positive cells. In contrast, if cells are harvested significantly more than 48 hr after challenge with a low titer virus, virus spread will manifest in a greater number of infected cells which will complicate experiments designed to determine or normalize virus titers.

Two harvesting options (A & B)

First, wash infected cells on the plate 1x with PBS (no ions)

Option A. Add 300 µl trypsin to each well, incubate at 37°C for no more than 5 minutes, and prepare 1.5 ml eppendorfs containing 1 ml of any media with serum to kill trypsin.

After trypsin incubation, break up remaining cell clumps in the 12-wells and transfer trypsinized cells to medium-containing eppendorfs and spin cells in eppi fuge at 7,000 rpm for 30 secs. Remove media, wash cells with PBS (no ions) once, and spin again. Re-suspend cell pellet in 4% paraformaldehyde.

Option B. Wash cells once more with PBS (no ions). Add 300 µl PBS/1 mM EDTA to each well and place on a shaking platform at room temp for 15 minutes. Prepare eppendorf tubes with 300 µl of 4% paraformaldehyde. With a blue tip, vigorously pipette to remove infected cells and place into eppendorfs with para fixative, vortex. Final concentration of 2% paraformaldehyde is sufficient.

Remove from BL-3 and keep on ice or at 4°C for at least 1 hr. If sensitivity isn't an issue, cells can be maintained, light-protected, this way for up to 48 hrs - the GFP is very stable. Fixation with paraformaldehyde will kill the virus. Outside the BL-3, it's still a good idea to handle all previously infected cell samples with gloves and decontaminate anything used in manipulating the samples (e.g. Pasteurs, FACS machine intake, etc.) with 10% bleach or EtOH.

After incubation on ice, spin cells in eppi fuge at 7,000 rpm for 30 secs, remove fixative. Resuspend cells in 200 µl PBS/2% serum.

Analyze by FACS for GFP expression. Expect an approx 20-fold shift in mean GFP-fluorescence of infected cells over non-infected.

Controls to consider:

1. Mock infected cells of each cell type tested.
2. Challenge each cell type tested with a HIV/VSV-G or HIV/Ampho Env pseudotype to demonstrate cells are healthy and transducible.
3. Challenge of GHOST parental cells with every virus being tested.

Last Updated June 22, 2017