

## NIH AIDS Reagent Program

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## **DATA SHEET**

Reagent:	HOS CD4+CCR3+ Cells
Catalog Number:	3316
Lot Number:	97153
Release Category:	C
Provided:	1 vial frozen cells.
Cell Type:	HOS (osteosarcoma) cells
Propagation Medium:	DMEM (containing 4.5 gm/L of glucose), 90%; fetal bovine serum 10%; supplement with 0.5-1.0 µg/ml puromycin. <b>NOTE:</b> CD4 expression decreases with long-term (~ 4 months) culture in the absence of selection. Every few months, the CD4 <sup>+</sup> cells can be selected for by using MPA medium (see attached). If desired, the cells can be maintained continuously in this CD4 selection medium.
Freeze Medium:	DMEM, 70%; fetal bovine serum, 20%; DMSO, 10%.
Growth Characteristics:	Thaw cells quickly at 37°C and immediately place them in 10 ml culture medium. Centrifuge at 400 X g to wash out DMSO, resuspend the cells in 10 ml fresh culture medium, and plate them onto a 10 cm <sup>2</sup> tissue culture dish. Cells normally require a minimum of 3-4 days to recover, but should be checked daily to see if they need to be split. Cells split 1:10 should become confluent after three days. Trypsinize and split at least twice a week; do not allow them to become over confluent.
Morphology:	Adherent, flat cells
Sterility:	Negative for mycoplasma, bacteria and fungi.
Description:	These are HOS cells expressing CD4 and CCR3.

## ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.

Special Characteristics:	cDNA encoding CCR3 was subcloned into the retroviral vector pBABE-puro. Amphotropic virus stocks were prepared by cotransfecting 293T cells with the resulting pBABE-puro construct, a VSV-G envelope expression vector, and pSV-gag-pol. $\psi$ renv <sup>-</sup> (Landau & Littman, 1992). Supernatants were collected after 48 hours and used to infect HOS CD4+ Cells (cat# 3313). After another 48 hours, cells were selected in medium containing 1 µg/ml puromycin. β-chemokine receptor expression can be maintained by culturing the cells in medium containing 0.5-1.0 µg/ml puromycin.
	confirmed by flow cytometry. Single cells were then individually sorted and propagated, with continued monitoring of expression of key receptors. Despite these efforts, the population remains heterogeneous for expression.
	Please also see HOS CD4+ Cells (cat# 3313) and other cells bearing various other $\beta$ -chemokine receptors (collectively cat#s 3314-3319)
	<u>Sample plasmid map</u> <u>Micophenolic Acid Medium</u>
	Alternate names: HOS-CD4-CCR3
Recommended Storage:	Liquid nitrogen.
Contributor:	Dr. Nathaniel Landau, Aaron Diamond AIDS Research Center, The Rockefeller University.
References:	Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR. Identification of a major co-receptor for primary isolates of HIV-1. <i>Nature</i> <b>381</b> :661-666, 1996.
	Landau NL, Littman DR. Packaging system for rapid production of murine leukemia virus vectors with variable tropism. <i>J Virol</i> <b>66</b> :5110-5113, 1992.
NOTE:	Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HOS CD4+CCR3+ Cells from Dr. Nathaniel Landau." Also include the references cited above in any publications.
	Patent pending. Limited to one aliquot per laboratory. Requests from commercial organizations must be directed to the New York University Office of Industrial Liaison at the following email address: <u>sadhana.chitale@nyumc.org</u> .
Last Updated	July 06, 2020

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