

DATA SHEET

For research use only. Not for use in humans.

Reagent:	Human Osteosarcoma (HOS) CD4+ CCR5+ Cells
Catalog Number:	ARP-3318
Lot Number:	200004
Provided:	Each vial of ARP-3318 contains approximately 3.14×10^6 cells in 0.6 mL of freeze medium. Post-thaw viability was 93%.
Cell Type:	ARP-3318 is an adherent, flat, human osteosarcoma (HOS) cell line.
Propagation Medium:	The recommended propagation medium is 90% DMEM (containing 4.5 gm/L glucose) supplemented with 10% fetal bovine serum and 1 µg/mL puromycin.
Freeze Medium:	The recommended freeze medium is Gibco™ Recovery™ Cell Culture Freezing Medium.
Growth Characteristics:	CD4 expression decreases with long-term (~ 4 months) culture in the absence of selection. Every few months, the CD4+ cells can be selected by adding mycophenolic acid (MPA) medium (40 µg per mL mycophenolic acid, 250 µg per mL xanthine, 13.5 µg per mL hypoxanthine and 10 mM HEPES; instructions provided on the BEI Resources webpage) to the growth medium. If desired, the cells can be maintained continuously in this CD4 selection medium. This cell line was cultured <i>in vitro</i>, and the expression of the receptors of interest was confirmed by flow cytometry. Single cells were then individually sorted and propagated, with continued monitoring of the expression of key receptors. Despite these efforts, the population remains heterogeneous for expression. ARP-3318 cells should be thawed quickly at 37°C, immediately placed in 10 mL of culture medium, and centrifuged at $400 \times g$ to remove the freeze medium. The cells should then be resuspended in 25 mL of fresh culture medium and plated onto a tissue culture dish or flask. 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 approximately 3 days. Trypsinize and split at least twice a week; do not allow them to become overconfluent.
Sterility:	Tests for bacteria, fungi and mycoplasma were negative.
Description:	ARP-3318, also referred to as HOS-CD4.CCR-5, are CD4- and CCR5-expressing HOS cells belonging to a set of cell lines that express various β-chemokine receptors, available as BEI Resources ARP-3313 through ARP-3319.
Special Characteristics:	cDNA encoding CCR5 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.ψ-env-. Supernatants were collected after 48 hours and used to infect HOS CD4+ cells (BEI Resources Catalog No. ARP-3313). After another 48 hours, cells were selected in medium containing 1 µg/mL puromycin. β-receptor expression can be maintained by culturing the cells in medium containing 0.5-1.0 µg/mL puromycin.
Recommended Storage:	Keep at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer.
Contributor:	Dr. Nathaniel Landau, Aaron Diamond AIDS Research Center, The Rockefeller University

- References:** Deng, H., et al. "Identification of a Major Co-Receptor for Primary Isolates of HIV-1." *Nature* 381 (1996): 661-666. PubMed: 8649511.
- Landau, N. L. and D. R. Littman. "Packaging System for Rapid Production of Murine Leukemia Virus Vectors with Variable Tropism." *J. Virol.* 66 (1992): 5110-5113. PubMed: 1321291.
- Citation:** Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Human Osteosarcoma (HOS) CD4+ CCR5+ Cells, ARP-3318." Also include the references cited in any publication.
- Biosafety Level: 2** Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories (BMBL). Current Edition. Washington, DC: U.S. Government Printing Office.
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