Sup-GGR (*Gaussia* GFP Reporter) Human Cell Line

Catalog No. HRP-20042

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For research use only. Not for use in humans.

Contributor:

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Manufacturer:

NIH HIV Reagent Program

Product Description:

HRP-20042 is a novel dual-indicator (fluorescent/enzymatic reporter) cell line which offers two different readouts to quantify human immunodeficiency virus type 1 (HIV-1) infection. The parental SupT1-CCR5 cell line harbors a pLenti6-CCR5 expression construct. A construct expressing both *Gaussia* luciferase and hrGFP (humanized Renilla GFP) in a Tat- and Rev-dependent manner was engineered into SupT1-CCR5 to create Sup-GGR cells. To produce Sup-GGR cells, parental SupT1-CCR5 cells were transduced with a lentiviral reporter vector, pNL-GGR-RRE (SA), and cloned by limiting dilution.[1,2] The Sup-GGR (*Gaussia* GFP Reporter) cell line supports the replication of both X4 and R5-tropic HIV as efficiently as its parental cell line, SupT1-CCR5, and allows repeated sampling without the need to terminate the culture.[1]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in 80% RPMI-1640 medium, 10% fetal bovine serum and 10% dimethyl sulfoxide (DMSO).

Packaging/Storage:

This product was packaged aseptically, in screw-capped plastic cryovials. It should be stored at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability. To ensure the highest level of viability, the vial should be thawed and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:

When handling frozen vials, it is highly recommended that protective gloves, lab coat and full-face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Thawing and Growth:

Prior to thawing the Sup-GGR cells, prepare growth medium (GM) for use. Sup-GGR cells are grown in RPMI-1640 medium (ATCC® 30-2001™) containing 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 milligrams per milliliter glucose and 1500 milligrams per liter sodium bicarbonate, supplemented with 10% fetal bovine serum (ATCC® 30-2020™). This GM is formulated for use with a 5% CO_2 in air atmosphere. Note: HRP-20042 is a suspension cell line. Do not discard floating cells.

Rapidly thaw the vial of cells in a 37°C water bath with gentle agitation. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol, and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 4 mL of GM in a centrifuge tube. Centrifuge the cell suspension at 150 × g for 8 to 10 minutes at 18 to 25°C. Discard the supernatant and resuspend the cell pellet in 10 mL of pre-warmed GM. Transfer the cell suspension into a 75 cm^2 tissue culture flask. Incubate the new culture at 37°C and 5% CO_2.

Sub-culture procedure.
Cultures can be maintained by the addition of fresh GM or replacement of GM. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 × 10^5 cells per milliliter. Maintain a cell density between 1 × 10^5 and 1 × 10^6 cells per milliliter. To replace the GM, centrifuge the cell suspension at 150 to 300 × g for 8 to 10 minutes at 18 to 25°C, remove the supernatant and resuspend the cell pellet in fresh pre-warmed GM to the desired cell density. Add fresh medium every 2 to 3 days, depending on cell density.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH HIV Reagent Program, NIAID, NIH: Sup-GGR (*Gaussia* GFP Reporter) Human Cell Line, HRP-20042, contributed by David G. Russell and David W. Gludish."

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. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the NIH HIV Reagent Program Material Transfer Agreement (MTA). The MTA is available on our Web site at www.hivreagentprogram.org.

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The GGR vector is claimed in U.S. Patent number 9,719,127 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.[2]

References:

1. Salasc, F., et al. “A Novel, Sensitive Dual-Indicator Cell Line for Detection and Quantification of Inducible, Replication-Competent Latent HIV-1 from Reservoir Cells.” Sci. Rep. 9 (2019): 19325. PubMed: 31852924.
2. Russell, D. G. and D. W. Gludish, Personal Communication.

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