HEK-TLR2^{YFP} Cell Line

Catalog No. NR-9316

This reagent is the property of the U.S. Government.

For research use only. Not for human use.

Contributor:

Dr. Douglas Golenbock, Professor, Infectious Diseases and Immunology, The University of Massachusetts Medical School, Worcester, Massachusetts

Product Description:

The HEK-TLR2^{YFP} cell line is derived from an adherent human embryonic kidney epithelial cell line (HEK-293) by stable transfection with a cDNA construct coding for a Yellow Fluorescent Protein (YFP)-Human Toll-Like Receptor 2 (TLR2) chimera.[1]

Material Provided:

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (90%) and DMSO (10%) cryo-preservatant. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual certificates of analysis for each product lot.

Packaging/Storage:

This product was packaged aseptically, in screw-capped plastic cryovials. It should be stored at cryogenic temperature (-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 insure 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 HEK-TLR2^{YFP} 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 HEK-TLR2^{YFP} cells, prepare growth medium (GM) for use. HEK-TLR2^{YFP} cells are grown in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen™ 11960-044) modified to contain 10% irradiated fetal bovine serum (FBS; Lonza 14-471F), 2 mM L-glutamine (Invitrogen™ 25030-081), 1 mM sodium pyruvate (Invitrogen™ 11360-070), and 1 mg/mL G418 (Invitrogen™ 11811-023). This GM is formulated for use with a 5% CO_2 in air atmosphere.

Rapidly thaw the vial of HEK-TLR2^{YFP} 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 pre-warmed (37°C for 15 to 30 minutes) GM in a centrifuge tube. Centrifuge the cell suspension at 125 to 200 X g for 8 to 10 minutes at 18–25°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. Replace the GM with fresh GM every 2–3 days and incubate until the cell sheet is approximately 80% confluent.

Sub-culture procedure. Aseptically remove the GM and discard. Briefly rinse the cell layer with 4 to 15 mL of Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS; Invitrogen™ 14190-144) to remove all traces of serum. Discard the PBS. Add 2 to 8 mL of 0.05% trypsin-EDTA (Invitrogen™ 25300-062) to the culture flask and incubate the flask at 37°C until cell layer is dispersed (usually within 3 minutes but no longer than 15 minutes). *Note: To avoid clumping, do not agitate the cells by hitting or shaking the flask.* Add an equal volume of GM and aspirate cells by gently pipetting. Perform a cell count and add appropriate aliquots of the cell suspension to new culture vessels at a sub-cultivation ratio of 1:2 to 1:6. Adjust the volume of GM to 15–20 mL for a 75 cm^2 flask. Incubate cultures at 37°C and 5% CO_2. Replace the GM with fresh GM every 2–3 days and incubate until the cell sheet is approximately 80% confluent.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: HEK-TLR2^{YFP} Cell Line, NR-9316."

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. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

This cell line has **NOT** been screened for hepatitis B virus, human immunodeficiency virus, or other adventitious agents. Cell lines derived from human tissue fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC® recommends that appropriate safety procedures[2,3] are used when handling all cell lines, especially those derived from human material.

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 BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government make any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Latz, E., et al. "Lipopolysaccharide Rapidly Traffics to and from the Golgi Apparatus with the Toll-like Receptor 4-MD-2-CD14 Complex in a Process that Is Distinct from the Initiation of Signal Transduction." J. Biol. Chem. 277 (2002): 47834–47843. PubMed: 12324469.
2. Caputo, J. L. "Biosafety Procedures in Cell Culture." J. Tissue Culture Methods 11 (1988): 223–227.
3. Fleming, D. O., J. H. Richardson, J. J. Tulis, and D. Vesley, eds. Laboratory Safety: Principles and Practice. 2nd ed. Washington, DC: ASM press, 1995.

Support Provided by NIAID

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898