

**Modification Form for Permit BIO-RR1-0033**

**Permit Holder: Michael Rieder**

**Approved Personnel**  
**(Please stroke out any personnel to be removed)**

James Liang  
Steven Thomson  
Kemi Adeyanju  
Jane Tucker

**Additional Personnel**  
**(Please list additional personnel here)**

Lauren Hanly

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
<b>Approved Microorganisms</b>		
<b>Approved Cells</b>	Human (primary), Human (established), Jurkat E6-1, Jurkat E6-1 transfected cells	HK-2
<b>Approved Use of Human Source Material</b>	Blood from Patients with adverse drug reactions	
<b>Approved GMO</b>	HIV	
<b>Approved use of Animals</b>		

\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

\*\* PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 1

Date of last Biohazardous Agents Registry Form: Feb 15, 2007

Signature of Permit Holder: 

BioSafety Officer(s): \_\_\_\_\_

Chair, Biohazards Subcommittee: \_\_\_\_\_

*Modification Form for Permit BIO-RRI-0033*

*Permit Holder: Michael Rieder*

Approved Toxin(s)

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Chair, Biohazards Subcommittee: \_\_\_\_\_

## Cell Biology

ATCC® Number: **CRL-2190™**  Price: **\$264.00**  
 Designations: HK-2 Depositors: RA Zager  
 Biosafety Level: 2 [Cells Contain Papilloma viral DNA sequences ] Shipped: frozen  
 Medium & Serum: See Propagation Growth Properties: adherent epithelial  
 Organism: *Homo sapiens* (human) Morphology:

Source: **Organ:** kidney, cortex  
**Tissue:** proximal tubule

Cellular Products: **Cell Type:** human papillomavirus 16 (HPV-16) transformed alkaline phosphatase; gamma glutamyltranspeptidase; leucine aminopeptidase; acid phosphatase; cytokeratin; alpha 3, beta 1 integrin; fibronectin

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Receptors: epidermal growth factor (EGF), expressed

Amelogenin: X,Y

CSF1PO: 13

D13S317: 9

D16S539: 11,12

DNA Profile (STR): D5S818: 12

D7S820: 10,11

THO1: 9

TPOX: 8,9

vWA: 17,18

Age: adult

Gender: male

HK-2 (human kidney 2) is a proximal tubular cell (PTC) line derived from normal kidney.

The cells were immortalized by transduction with human papilloma virus 16 (HPV-16) E6/E7 genes.

The recombinant retrovirus vector pLXSN 16 E6/E7 containing the HPV-16 E6/E7 genes was used to transfect the ectotropic packaging cell line Psi-2.

Virus produced by the Psi-2 cells was used to infect the amphotropic packaging cell line PA317 (see ATCC CRL-9078).

Virus produced by the PA317 cells was used to transduce primary PTCs.

Although pLXSN 16 E6/E7 also confers resistance to neomycin, selection in G418 was not used to isolate transduced clones.

The cell line appears to be derived from a single cell based on Southern and FISH analysis.

The E6/E7 genes are present in the HK-2 genome as determined by PCR.

- The cells retain a phenotype indicative of well differentiated PTCs. They are positive for alkaline phosphatase, gamma glutamyltranspeptidase, leucine aminopeptidase, acid phosphatase, cytokeratin, alpha 3,beta 1 integrin, and fibronectin. The cells are negative for factor VIII related antigen, 6.19 antigen and CALLA endopeptidase.
- Comments:** HK-2 cells retain functional characteristics of proximal tubular epithelium such as Na<sup>+</sup> dependent / phlorizin sensitive sugar transport and adenylate cyclase responsiveness to parathyroid, but not to antidiuretic hormone. The cells are capable of gluconeogenesis as evidenced by their ability to make and store glycogen. HK-2 cells are anchorage dependent. The cells will not grow in methylcellulose, soft agar or suspension. HK-2 cells can reproduce experimental results obtained with freshly isolated PTCs.
- Propagation:** **ATCC complete growth medium:** The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line (bovine pituitary extract (BPE) and human recombinant epidermal growth factor (EGF). To make the complete growth medium, you will need to add the following components to the base medium:
- 0.05 mg/ml BPE - provided with the K-SFM kit
  - 5 ng/ml EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.
- Atmosphere:** air, 95%; carbon dioxide (CO<sub>2</sub>), 5%  
**Temperature:** 37.0°C  
**Growth Conditions:** Cell growth is dependent on epidermal growth factor. The cells should not be allowed to become confluent. Subculture at 80% of confluence.
- Protocol:**
1. Remove and discard culture medium.
  2. Briefly rinse the cell layer with 0.05% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
  3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).  
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
  4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
  5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
  6. Incubate cultures at 37°C.
- Subculturing:**

**Subcultivation Ratio:** A subcultivation ratio of 1:4 is recommended

**Medium Renewal:** Every 2 to 3 days

**Freeze medium:** Complete growth medium supplemented with 7.5% (v/v)

Preservation: DMSO

**Storage temperature:** liquid nitrogen vapor phase

References:

22466: Ryan MJ, et al. HK-2: an immortalized proximal tubule epithelial cell line from normal adult human kidney. *Kidney Int.* 45: 48-57, 1994. PubMed: [8127021](#)

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These cells will be cultured and then treated with the chemotherapy agent ifosfamide, as well as several antioxidants. This will be done in order to assess the nephrotoxic effects of ifosfamide and the ability of antioxidants to attenuate it.

These cells will be cultured and treated with the chemotherapy agent ifosfamide, as well as several antioxidants.