HYBRIDOMAS AND CELL LINES DEPOSIT FORM – Part 2 of 2

INSTRUCTIONS TO DEPOSITOR: Please fill in the **relevant and available information** about each deposit. Since this form covers multiple collections please use NA in any required fields that do not apply to your deposited material. Required fields are highlighted in yellow. **Please return this form electronically prior to shipping the Material. A printed copy should also be included with the shipment.** Additional information, references or pages maybe attached as needed. This information helps us better characterize and preserve your deposit.

The "**MATERIAL**" subject to this Deposit Form is: ◯ Cell line ◯ Hybridoma

Complete accepted name of material: HEK 293T-ACE2 cells

Genus: Homo sapiens

Species: _____

1. BACKGROUND INFORMATION

a. This material was originated by (include institution/state/country): Bloom lab, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

b. Date of origination of material: March 15, 2020

c. Origination source:

i) Did this material originate from:

A human subject? ☐ Yes ☑ No

An animal subject? ☐ Yes ☑ No If yes, please identify: _____

An animal "wildlife" ☐ Yes ☑ No If yes, please identify: _____

If from "wildlife", please indicate whether bred in the wild ☐ or in captivity ☐.

If from "wildlife", and bred in the wild, indicate permit number: _____

If from "wildlife", and bred in captivity, indicate animal number: _____

Please attach breeder's statement.

ii) If cell line(s), please describe:

Cell type: HEK 293T Organ/Tissue: _____

Cytopathological state: _____ Ethnicity/Breed: _____

Age: _____ Sex: _____ Blood type: _____

Immortalization method: _____

Clinical disease or symptoms exhibited by host, if applicable: N/A

d. If you did not originate this material, indicate your source and the material's prior history:

DEPOSITOR ← ATCC ← _____ ←

_____ ← _____ ← _____

e. Citation(s): *Please cite or attach a copy of or relevant references*. _____

f. Reason for deposit (please select one of the following):

☐ Requested by ATCC

☑ Other(s), please specify: Enable pseudotype neutralization assays w SARS-CoV-2 Spike

g. Was this material isolated/generated using any U.S. Government funding? ☑ Yes ☐ No If yes, please indicate all the funding sources, agencies, and/or programs: NIH / NIAID grants R01AI141707 and F30AI149928

h. Intended use or applications for this cell line(s) (e.g. host for protein expression, host for intracellular pathogen, vector cell/molecular biology etc.): Target cell for viruses using ACE2 as cellular receptor, such as SARS-CoV-2.

2. CHARACTERISTICS OF MATERIAL:

a. Passage number if known: 7

b. Life expectancy in terms of number of subcultivations before senescence occurs, if known: NA

c. Growth mode (i.e. suspension, adherent or semi-adherent cell line(s), other subculturing requirements): Adherent

d. Population doublings (PDLs), etc.): _____

e. Clonal method (if any) including selection method and stability: _____

f. Have there been any important changes in culture media or methods since origination/isolation? ☐ Yes ☑ No
If yes, please describe: We culture just like standard 293T cells.

g. Describe any quality control tests and results of tests (STR, COI analysis, mycoplasma, or other adventitious pathogen screening): ACE2 expression was verified by flow cytometry.

h. Have these cells been genetically modified? ☑ Yes ☐ No If yes, please describe how: These cells have been lentivirally transduced to express human ACE2.

i. Genotype and related NCBI accession number(s): _____

j. If hybridoma, please describe: _____

i) Fusion partner (designation, cell line(s), species, etc.): _____

ii) Route of immunization in vivo or in vitro (footpad intracapular, intreaperitoneal, etc.): n/a

iii) Antigen: n/a

◯ intact cell ◯ crude extract ◯ purified protein ◯ cell lysate
◯ purified recombinant preparation ◯ synthetically prepared ◯ other:_____

Conjugated (BSA, Ovalbumin): _____ Age of immunized subject: _____

Adjuvant: _____ Screening assay: _____

iv) Antibody: n/a

Specificity: _____

Cross-reactivity (if any):_____

Ig Class: _____ Subclass: _____

Light chain: ◯ lambda ◯ kappa ◯ Unknown

v) Special properties, characteristics, or use:

Is it neutralizing?	☐ Yes	☐ No	☐ Unknown
Can it bind to both denatured and native protein?	☐ Yes	☐ No	☐ Unknown
Can it be labeled or radio-labeled without losing specificity?	☐ Yes	☐ No	☐ Unknown

Can it be used for:

Western blots?	☐ Yes	☐ No	☐ Unknown	Titer _____
Immunoprecipitation?	☐ Yes	☐ No	☐ Unknown	Titer _____
Flow cytometry?	☐ Yes	☐ No	☐ Unknown	Titer _____
Immunocytochemistry?	☐ Yes	☐ No	☐ Unknown	Titer _____
Radioimmunoassays?	☐ Yes	☐ No	☐ Unknown	Titer _____
ELISA	☐ Yes	☐ No	☐ Unknown	Titer _____
Neutralizing assays	☐ Yes	☐ No	☐ Unknown	Titer _____
Immunohistochemistry?	☐ Yes	☐ No	☐ Unknown	Titer _____
Immunofluoresence?	☐ Yes	☐ No	☐ Unknown	Titer _____

3. PROPAGATION AND PRESERVATION CONDITIONS

a. Has the cell line(s) been screened for the presence of adventitious agents? ☐ Yes ☑ No

If yes, please specify test methods and results:

_____

b. Recommended propagation medium and procedure:

i) Medium (include concentration of additives/supplements or antibiotics, please attach formula if uncommon media):

DMEM + 10% FBS + 2 mM L-glutamine + 100 U/ml penicillin + 100 ug/ml streptomycin

ii) Conditions (atmosphere, number of days of incubation to obtain log phase growth, etc.):
5% CO2

iii) Subcultivation procedure (including frequency of subculture, special cultivation procedures):
Trypsinize with 0.25% trypsin / EDTA then split ~1:8 every ~3 days

c. Recommended storage and preservation conditions:

Temperature and form (lyophilized at 4˚C, frozen at -20˚C, etc.): Frozen at <= -80C

Cryoprotectant name and formula: Growth media + 20% FBS + 10% DMSO

Other additional info: _____

4. FINAL PREPATION OF MATERIAL AS SUBMITTED

a. State how the cell line(s) will be provided (i.e. plastic cryopreservation vial, glass vial, etc.):
Four plastic cryopreservation vials (1 mL each)

b. Give the formulation for the medium, cryoprotectant, etc. in which the cell line(s) is preserved (if different from recommendations above):
Same as described in 3c

i) Indicate if any carcinogenic chemicals were used:
_____

ii) Include the manufacturer and country of origin:
Gemini Bioproducts

iii) Identify any reagents of plant/animal origin used to cultivate this strain (serum, growth factors, etc.):
FBS

c. Number of vials being transferred: _____

d. Label designation on vials being transferred: 293T-hACE2

5. SAFETY AND REGULATORY INFORMATION

a. Is this material hazardous to:

i) Humans? ☐ Yes ☑ No Animals? ☐ Yes ☑ No Plants? ☐ Yes ☑ No ☐ Unknown?

If yes, what is the Biosafety Level (BSL) required to handle it? (Refer to Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publications No. (CDC) 93-8395 U.S. Department of Health and Human Services. The complete text is available at www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm): ○ **BSL 1** ○ **BSL 2** ○ **BSL 3**

ii) Is this cell line(s) listed on the U.S. Government's Select Agent list? ☐ Yes ☑ No

iii) Is this material of human origin: ☑ Yes ☐ No

If yes, was an IRB-approved consent form obtained: ☐ Yes ☐ No ☑ Not applicable

If yes, provide the IRB number: _____

b. Has this material been transformed with any hazardous agents? ☐ Yes ☑ No If yes, please specify:
_____

HYBRIDOMAS AND CELL LINES DEPOSIT FORM – Part 2 of 2

c. Does this strain require special permits (e.g. APHIS, CDC, Form 2)? ☐ Yes ☑ No If yes, please specify:

_____

Additional Comments:

_____

_____

_____