When finished, please email reply to: Brian Dattilo, PhD

Email: [bdattilo@waisman.wisc.edu](mailto:bdattilo@waisman.wisc.edu)

|  |  |
| --- | --- |
| Today’s Date |  |
| Principal Investigator or Client |  |
| Company or Institution |  |
| Product Name |  |
| Services Requested | Process Development  Assay Development  Cell Banking  cGMP Manufacture  Aseptic Fill |
| Quantity of virus desired (infectious vs. viral particles) |  |
| What agency will this product be regulated by? | FDA  EMEA  Other |
| Date Desired |  |

**Note: Waisman Biomanufacturing has a GMP produced and tested HEK293 cell bank for use in viral vector production. If this cell line will not be used, appropriate cells and Master/Working Cell Bank production and testing should be described.**

# How did you hear about Waisman Biomanufacturing?

# Product and Intended Use

## Please briefly describe the identity of your transgene.

## What AAV serotype do you require?

## What AAV production technology do you use?

HEK293 cell transfection

Baculovirus in insect cells

Other

## This product is intended for use in (check all that apply):

Research only and not for use in animals or humans

Animal /tox studies

Human clinical trials:

Phase I

Phase II

Phase III

## If used in human clinical trials, this product will be used for:

*Ex-vivo* applications (cell transfection/culture)

Direct injection

## What indication is this product for?

# Safety Information

## Is this product a select agent?

Yes

No

## What biosafety level?

BSL-1

BSL-2

BSL-3

# Manufacturing Information

## Describe the virus construct (restriction map, inserts, etc.) (attach).

## Product Quality

Table 1 lists commonly used release specifications. Please check criteria you currently use or would like to use and fill in required specification if possible.

Table 1

| **Test** | **Methods / Comments** | **Typical Starting Specification** | **Desired** | **Requested Specification** |
| --- | --- | --- | --- | --- |
| Identity - Protein | SDS-PAGE | Pattern matches standard |  |  |
| Replication Competent (wild-type) AAV | Infectious Center Assay | < 1 per 3 x 1010 PU |  |  |
| Host Cell DNA | PCR assay for host protein | WHO < 100 pg/dose |  |  |
| Host Cell Protein | HCP ELISA (Cygnus) |  |  |  |
| Residual Benzonase  Other processing agents | Benzonase ELISA  BSA ELISA |  |  |  |
| Sterility | Sterility test | Pass |  |  |
| Mycoplasma | FDA PTC mycoplasma assay | Pass |  |  |
| In vitro adventitious agent testing | Testing on 3 cell lines | Pass |  |  |
| Endotoxin | LAL - kinetic turbidometric | < 5.0 EU/kg/dose |  |  |
| Virus Titer | qPCR for transgene |  |  | vg/ml |
| Infectivity Assay | limiting dilution in C12 or other appropriate cell line |  |  |  |
| *Functional Potency Assay* | *Please describe below (e.g. in vitro transduction/ELISA)* | *TBD* |  |  |

*Describe activity assay or any other assays that need development here, if necessary. Will these be performed by Customer or transferred to Waisman?*

## Do you have an executed batch record for this process?

Yes

No

## Describe what will you be providing as starting material? (E.g. Master Cell Bank, non-GMP plasmids, etc.)

## If applicable:

Please describe the cell substrate used for manufacturing if different from Waisman’s HEK293 adherent or suspension line.

## Outline prefered production steps (use attachments if needed).

Please note any convenient hold/storage points in the process and storage conditions (e.g. temperature, medium, etc).

Please list any specialized raw materials or vendors for your existing production process. Please note any special testing that is required for raw materials (e.g. cell culture testing of serum lots).

# Master Cell Bank

## If not provided, do you need a Master Cell Bank?

Yes

No, one will be provided

No, we will use Waisman’s HEK293 bank

If yes, how large of a bank (200-300 vial is typical)?

## If provided, has your cell line and/or tissue source undergone appropriate testing for adventitious agents? (Note: we cannot accept cell lines that have not undergone required testing for adventitious agents.)

# Plasmids

## If using a plasmid system, what plasmids will you provide? If using a non-3-plasmid system (e.g. both rep/cap and Ad helper on same plasmid, describe in ‘other’).

Transgene Plasmid (with ITRs)

AAV packaging plasmid (providing rep/cap genes)

Ad helper plasmid (providing adenovirus helper genes)

Other

## Please provide general information on the packaging vector(s) and transgene vector including restriction map, selectable markers, transgene(s), and regulatory components (promoter, other 5’ and 3’ inserts).

## Have the entire plasmids and inserts been sequenced and characterized? Are the plasmids currently produced from a characterized Master Cell Bank or is one necessary?

# Viruses

## If using a baculovirus system, what will you provide as starting material (e.g. SF9 bank, baculovirus seed bank, non-GMP baculovirus vectors)?

# Process Development

## What stage is process development needed (fermentation / cell culture, purification, etc)?

## What improvements are you targeting (yield, activity, purity, scalability, etc)?

# Viral Production

## If using HEK293 or other cell transfection, do you use adherent or suspension cell culture?

## What is your typical yield in viral particles per cell?

## What assays or methods need to be performed during viral production and harvest?

## What assays or methods are used to determine when to harvest?

## What is the optimum viral production time after transfection?

## At harvest, what percentage of viral product is in the media? What percentage is in the cells? Should the production process include recovery from both media and infected cells?

## How is the viral product harvested after propagation? Centrifugation, microfiltration, ultrafiltration, other.

# Down-Stream Processing

## Describe any special steps prefered before starting downstream processing? Cell lysis, RNA or host DNA removal, etc.

## What chromatographic, filtration, or other purification steps are prefered to reach purified viral vector. Please describe in detail.

# Quality Control

## Earlier in this document (Table 1) were common assays that are used for release testing of proteins. Please check which assays you’d like done and indicate your proposed acceptance criteria (Note: acceptance criteria should be based on at least one qualification lot and ideally three qualification lots). Typical acceptance criteria is listed, please indicate if you have different requirements. Also, please indicate which (if any) assays you would like qualified (for Phase 1-2, assay qualification is usually limited to assays that effect labeling such as concentration or potency).

## What assays are used to verify viral activity and/or function? Is a validated potency assay available? Are there any special product assays available (e.g. transduction assay)?

1. Can you provide us with assays for measuring the identity and concentration of the transgene product (ELISA, Western Blot, etc)?

# Bulk Storage

## What are your packaging and storage specifications for final purified bulk product?

# Dispensing (optional)

## What is the total number of vials or other containers required (note: include enough excess for long term storage, sampling, and stability testing)?

## Formulation of vehicle/adjuvant?

## Do you require a placebo fill (how many, if so)?

## What are the specifications for fill volume (total volume +/- range) and concentration (concentration +/- range)?

## Are you aware of any problems with material compatibility - containers, tubing, glass, filters, etc.?

## Do you have information on the preferred storage conditions and stability of the product in bulk form, in process, and in final state?

# Please provide any other information or clarification