

Mail Code L584 - 505 N.W. 185th Avenue, Beaverton, OR 97006 – Lab Tel: 503-346-5111,

Website: <https://www.ohsu.edu/onprc/molecular-virology-core>

**AAV Neutralizing Antibody Assay Request Form**

*Please e-mail to completed form to* carrolju@ohsu.edu *and* disseng@ohsu.edu

**Date:** Click here to enter a date.

**Contact Information**

**Principal Investigator:** Click here to enter text.

**Email Address:** Click here to enter text.

**Phone Number:** Click here to enter text.

**Laboratory Contact:** Click here to enter text.

**Email Address:** Click here to enter text.

**Phone Number:** Click here to enter text.

**Institution:** Click here to enter text.

**Department Name and Code:** Click here to enter text.

**Mail code:** Click here to enter text.

**Shipping Address:**  Click here to enter text.

 Click here to enter text.

 Click here to enter text.

 Click here to enter text.

**Project Alias Number (for internal users):** Click here to enter text.

**FAID (for internal users):**  Click here to enter text.

**Name of Fiscal Authority (for internal users):** Click here to enter text.

**Sample Information**

1. **Number of samples:**
2. **Source of sample (eg Monkey (please specify species of NHP)/Mouse etc.):**
3. **Sample type:**

**Serum** [ ] Yes [ ] No  **Plasma** [ ] Yes [ ] No

1. **Sample Volume (For monkey samples minimum of 200 ul is required per sample per assay per serotype ;300ul preferable):**
2. **Please indicate the sample ID numbers and blood draw dates :**

**AAV Neutralizing Antibody Assay Information**

1. **Please indicate the AAV serotype to be tested in the neutralizing antibody assay (e.g., AAV1, AAV2, etc.):**

1. **Please indicate the type of neutralizing assay desired:**

**Screening Assay**

**(to screen animals for NAb presence/absence; 3-dilution range)** [ ] Yes [ ] No

**ID50 Assay (full dilution range)** [ ] Yes [ ] No

1. **Have the samples been tested before for Neutralizing Antibodies? If so, against which AAV serotype?**
2. **Have the samples been tested before for binding Antibodies (e.g. ELISA). If so, against which AAV serotype?**

**General Assay Information:**

**Samples:**

* All samples are heat inactivated at 56° C for 1h to inactivate complement.
* For the screening assay, samples will be tested for neutralizing antibodies at three dilutions (1:5, 1:20, 1:80)
* For the ID50 assay, samples will be tested for neutralizing antibodies starting at a 1:5 dilution for monkey samples and 1:50 dilutions for mouse samples. Serial 2-fold dilutions, with a total of 11 dilutions and 2 replicates per sample will be used for ID50 determination

**AAV Neutralizing antibody assay:**

Using the luciferase reporter system we have developed a sensitive and easily quantifiable AAV Neutralizing Antibody assays, currently available for AAV serotypes 1, 2, 8 and 9. Our virus neutralization assays test the potential ability of an antibody to block the AAV luciferase reporter virus transduction into susceptible cells. The assays are carried out in 96 well format with 5 x 10^4 cells per well. (For AAV9 we use CHO-Lec2 cells and for all other AAV serotypes we use CHO-K1 cells). For the assay, various dilutions of serum (antibodies) are pre-incubated with 1E+9GC of AAV reporter virus for 1 h at 37°C and are then added to cells that have been infected with Adeno Helper virus. After 48 h the Promega Bright-Glo substrate is added to the cells and luciferase expression is quantitated using the Biotek Synergy Mx luminometer. For the ID50 assay, the ID50 or the neutralizing antibody titer at 50% inhibition of virus transduction is calculated based on the global fitting of the data obtained from each of the 11 serum (antibody) dilutions and is based on the 4 parameter formula:

 $y=\frac{a-d}{[1+\left(x/c\right)^{b} ]}+d$

a = (theoretical) response at dilution = 0

b = measure of the slope of the curve at its inflection point

c = value of x at inflection point (ID50)

d = (theoretical) response at infinite dilution

x = dilution

y = response (RLU)

The data is fitted using the Gen5 software.

|  |  |
| --- | --- |
| **To:** Julie Carroll/Greg Dissen ONPRC,  Molecular Virology Core Mail Code L584 Research Building 046 505 NW 185th Avenue Beaverton, OR 97006 Phone: 503-346-5111 | **From:** **(PLEASE PROVIDE SENDERS NAME, SHIPPING ADDRESS AND PHONE NUMBERS)** **RESPONSIBLE PERSON**  **FOR THIS SHIPMENT** |
| Emergency Contact Number: 503-936-6103 |

**For external (non-OHSU) users**, materials will be shipped and received via FedEx using the provided address and account number. An email will be sent prior to shipment to the requesting user confirming the day of shipment and the tracking information once available. Please inquire for specific routine or custom service requests, and we can generate a quote for you. External billing proceeds through the ONPRC business office. Note that center guidelines require that external users supply us with a purchase order (PO) before initiating a service. After the work is completed, external users will receive an invoice for review and the supplied PO will be billed.