**Order of AD drug screen Assay**

Date： Jul 22th 2019

NO.: 20190722

1. **Information from the client**

**Table 1 Information from the client**

|  |  |  |  |
| --- | --- | --- | --- |
| Name: |  | Institute/ university:  |  |
| PI (If different from above): |  | Department： |  |
| Tel: |  | E-mail: |  |
| Delivery Address: |  |
| Billing Address (If different from above): |  |

Is the client willing to share his/her name, lab, and email address with SUNY’s future clients if they inquire? \_\_\_\_\_\_\_

How did the client learn about SunyBiotech? \_\_\_\_\_\_\_\_\_

1. Email B) Mail C) Website D) From colleagues or friends

E) Other

(If the client chooses D, please write down the referrer name and institute and SUNY will apply 5% OFF to both the client and the client’s referrer. But please kindly note the 5% OFF is only applicable when the referrer comes from other labs.)

1. **About this service**

The purpose of this assay is to compare the paralysis incidence of Alzheimer disease model CL2006 under drug treatment. For every drug treatment, at least 80/240 worms will be tested in one/ three replicate(s), data of 67/200 tested worms will be provided.

1. **Information about the tested drug**
	1. Please complete the information of the treated drugs in the Table 2 and send all drugs powder to SUNY.
	2. Is the drug sterilized or not? ( for example: yes, no). If not, please provide the sterilization method . If the syringe driven filtration is required, please choose the pore size of the syringe driven filter (0.22µm or 0.45µm).
	3. The drug should be added into for *C. elegans* culturing. (for example: **NGM, OP50**)

**Table 2 Information of the drugs**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Drug name/ NO. | Concentration of the stock | Storage condition | Solvent | working concentration  | Working volume |
| 1 |  |  |  |  |  |  |
| 2 |  |  |  |  |  |  |
| 3 |  |  |  |  |  |  |

1. **Method/ protocol**

**Prepare samples**

* 1. Dissolve drugs or chemicals in the solvent.
	2. Prepare NGM plates
		1. For the control plates, mix the solvent with OP50 and seed them on the NGM plates before using.
		2. For the experimental plates, mix the drugs with OP50 and seed them on the NGM plate, or drop the drug directly on the OP50 lawn.

**Prepare Synchronized animals**

* 1. Culture CL2006 worms in 16 °C incubator
	2. Collect one day CL2006 adults, transfer 20-30 adults into **control NGM plate and experimental NGM plate** with drugs, let them lay eggs for 2 hours in 16 °C.
	3. Put all the synchronized eggs in the same 20 °C incubator until the synchronized CL2006 are at L4 stage.

**Score animals for paralysis incidence**

1. Transfer 30 L4 worms to each **control NGM plate and experimental NGM plate**, 3 plates for every assay. Keep the worms in 20 °C incubator. Record the number of paralysis CL2006 every day or every two days.
2. Transfer the worms everyday (during the egg laying period) to avoid the influence of the progeny.
3. **Dead/Paralysis** worms would be **counted and removed** from the plate. Worms that crawl out of plates will not be counted.
4. Animals are considered paralysis if they failed to move their bodies when touched and exhibited a “halo” of cleared bacterial lawn by moved their heads only.
5. **Duration and Cost of the service**
6. Usually it will take about 4-6 weeks to do the drug screen experiment. The turnaround time will be extended when the drug delays the growth and reproduction of CL2006. SUNY will inform the client when the turnaround needs to be extended.
7. Turnaround time is defined as the time from when an order is accepted to when it is shipped. It does not include waiting time for any materials that the client needs to supply.
8. The price is $500/1400 for every/ three replicate(s) of one working concentration. The price for this order would be for treatments with replicates.
9. SUNY will start the experiment after the client confirms the order. If the client needs to modify any order that has already been confirmed and started production, this may incur an additional charge.
10. **Products SUNY provide**
11. Raw data of every assay: the number of paralyzed and dead CL2006.
12. Paralysis incidence of every assay.
13. **How and when to pay**
	1. T/T (Telegraphic Transfer)
	2. If it is client’s first time placing an order with SUNY and the total amount is no more than 3000 USD, no pre-payment is required. Otherwise, SUNY will require a pre-payment that equals to 50% of the total amount of client’s order prior to providing the service.
14. **Force Majeure**

SunyBiotech shall not be liable for damages or delays by causes beyond its reasonable control such as: lightning, fire, and explosion; pest damage; strikes or labor disputes; floods; war, terrorism, civil disturbances, and acts of civil or military authorities or the public enemy; inability to secure transportation facilities or fuel that is beyond its reasonable control; energy shortages; acts or omissions of communications carriers; or other causes beyond the reasonable control whether or not similar to the foregoing.

1. **Reference**

[1] Link, C. D. (1995). Expression of human beta-amyloid peptide in transgenic Caenorhabditis elegans. Proceedings of the National Academy of Sciences, 92(20), 9368–9372. doi:10.1073/pnas.92.20.9368

[2] Dostal, V., & Link, C. D. (2010). Assaying β-amyloid Toxicity using a Transgenic C. elegans Model. Journal of Visualized Experiments, (44). doi:10.3791/2252

[3] Regitz, C., Marie Dußling, L., & Wenzel, U. (2014). Amyloid-beta (Aβ1-42)-induced paralysis inCaenorhabditis elegansis inhibited by the polyphenol quercetin through activation of protein degradation pathways. Molecular Nutrition & Food Research, 58(10), 1931–1940. doi:10.1002/mnfr.201400014