
**Gangliosides for standardizing and increasing the sensitivity of cells to Botulinum
neurotoxins in *in vitro* test systems**

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The present invention pertains to a method for standardizing the sensitivity of induced pluripotent stem cell (iPS)-derived neurons to a neurotoxin polypeptide, comprising the steps of: a) cultivating different batches of induced pluripotent stem cell-derived neurons in a cell culture medium comprising GT1b for at least 3 hours;
10 b) contacting the different batches of induced pluripotent stem cell-derived neurons of step a) with a neurotoxin polypeptide; c) cultivating the different batches of induced pluripotent stem cell-derived neurons of step b) for at least 24 hours in the presence of GT1b under conditions which allow for the neurotoxin polypeptide to exert its biological activity, thereby standardizing the sensitivity of the induced
15 pluripotent stem cell-derived neurons to a neurotoxin polypeptide. The invention further relates to a method for the generation of induced pluripotent stem cell-derived neurons having a standardized sensitivity to a neurotoxin polypeptide, comprising the steps of: a) providing different batches of induced pluripotent stem cell-derived neurons; b) cultivating the different batches of induced pluripotent stem
20 cell-derived neurons of step a) in a cell culture medium comprising GT1b for at least 3 hours, thereby standardizing the sensitivity of the induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide. In addition, encompassed by the present invention is a method for determining the biological activity of a neurotoxin polypeptide, comprising the steps of:

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a) cultivating induced pluripotent stem cell-derived neurons in a cell culture medium comprising GT1b for at least 3 hours;

b) contacting the induced pluripotent stem cell-derived neurons of step a) with a neurotoxin polypeptide;

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c) cultivating the induced pluripotent stem cell-derived neurons of step b) for at least 24 hours in the presence of GT1b under conditions which allow for the neurotoxin polypeptide to exert its biological activity; and

d) determining the biological activity of the neurotoxin polypeptide in said cells, wherein different batches of induced pluripotent stem cell-derived neurons are used.

Finally, the invention relates to the use of GT1b for a) standardizing the sensitivity of different batches of induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide; or b) reducing the variability of the sensitivity of different batches of induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide.

- b) contacting the induced pluripotent stem cell-derived neurons of step a) with a neurotoxin polypeptide;
 - c) cultivating the induced pluripotent stem cell-derived neurons of step b) for at least 24 hours in the presence of GT1b under conditions which allow for the neurotoxin polypeptide to exert its biological activity; and
 - d) determining the biological activity of the neurotoxin polypeptide in said cells, wherein different batches of induced pluripotent stem cell-derived neurons are used.
5. The method of claim 1 or 3, further comprising determining the biological activity of the neurotoxin polypeptide in the different batches of induced pluripotent stem cell-derived neurons.
 6. The method of any of claims 1 to 3, wherein standardizing the sensitivity is a reduction in the variability of the sensitivity of the different batches of induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide, in comparison to control batches of induced pluripotent stem cell-derived neurons treated without GT1b.
 7. The method of claim 6, wherein the reduction in the variability of the sensitivity of the different batches of induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide is an at least 1.5-fold or at least 2-fold reduction.
 8. The method of any of claims 1 to 7, wherein the induced pluripotent stem cell-derived neurons are human induced pluripotent stem cell-derived neurons, preferably iCell[®] neurons (Cellular Dynamics International).
 9. The method of any of claims 1 to 3 and 5 to 8, wherein the different batches of induced pluripotent stem cell-derived neurons differ in the number of passages, the number of freeze/thaw cycles, the cultivation conditions, the storage time, the growth time, the differentiation conditions, or combinations thereof.
 10. The method of claim 1, 2 or 4, wherein the cell culture medium comprises Neurobasal medium, B27 Supplement (2%), and Glutamin or Glutamax (1%).

11. The method of any of claims 1 to 10, wherein GT1b is added in a concentration of 1 to 300 μM , preferably 30 μM .
12. The method of any of claims 1 to 11, wherein the neurotoxin polypeptide is BoNT/A, BoNT/B, BoNT/C1, BoNT/D, BoNT/E, BoNT/F, BoNT/G, BoNT/H or TeNT, or a subtype thereof.
13. The method of claim 4 or 5, wherein the biological activity of the neurotoxin polypeptide is determined by quantification of the neurotoxin-cleaved substrate by Immuno-Western blot analysis, SDS-PAGE Immunoblot analysis or ELISA.
14. Use of GT1b for
 - a) standardizing the sensitivity of different batches of induced pluripotent stem cell-derived neurons to a neurotoxin polypeptide; or
 - b) reducing the variability of the sensitivity of different batches of induced pluripotent stem cell-derived neurons to a Neurotoxin polypeptide.