The Method of the Body Bending Assay Using *Caenorhabditis elegans*

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[Abstract] This protocol is useful to obtain clear and repeatable data to know the motor function of a worm by counting the number of body thrash in M9 buffer. The thrashing assay is useful for observation of the effect of loss of motor neurons such as VA or VB neuron.

Materials and Reagents

1. KH_2PO_4 (Wako Chemicals USA, catalog number: 169-04245)
2. Na_2HPO_4 (Wako Chemicals USA, catalog number: 197-02865)
3. NaCl (Wako Chemicals USA, catalog number: 191-01665)
4. M9 buffer (see Recipes)

Equipment

1. Sterile NGM agar plate with a 35 mm diameter

Procedure

Fill sterile NGM agar plates with a 35 mm diameter with 1 ml of M9 buffer. Before the start of this assay, put a worm on a sterile NGM agar plate without bacteria and allow it to crawl freely to remove the agglomerated bacteria from the worm. After examining whether the bacteria were removed by visually, put a worm (4-day-old) into the buffer and allow it to swim freely for 1 min to be accustomed to the environment.

Then count the number of thrash for 1 min (Nawa *et al*., 2012). Count at least 10 worms for each assay.

Note: A movement of the worm that swings its head and/or tail to the same side is counted as one thrash. A movie of body-bending worms makes counting more correct and easy. The bacteria that are served as food for worms should be removed as completely as possible because they affect the worm's movement.

Recipes

1. M9 buffer (Brenner, 1974)

Acknowledgments

This protocol is adapted from Brenner (1974) and previously used in Nawa *et al.* (2012).

References

1. Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77(1): 71-94.
2. Nawa, M., Kage-Nakadai, E., Aiso, S., Okamoto, K., Mitani, S. and Matsuoka, M. (2012). Reduced expression of BTBD10, an Akt activator, leads to motor neuron death. *Cell Death Differ* 19(8): 1398-1407.

