Application Note

PG-003 (TrkB Agonist Peptide)

PeptiGrowth

Effect of TrkB Agonist Peptide (Product Code: PG-003) on Neurite Outgrowth of Human Neuroblastoma Cell (SH-SY5Y)

Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that binds specifically to TrkB receptor on the target cell surface. It is regarded to be essential for nerve cell growth, survival, proliferation, and synaptic function control. Its expression in the brain and peripheral nervous system has been already identified [1] and proposed to play an important role in the maintenance of neuronal function. Its reduction is associated with development of various neurological diseases including dementia, suggesting its usefulness in studying the mechanisms of their pathogenesis [2, 3].

Neurite outgrowth is known to be enhanced by the addition of neurotrophic factors such as BDNF (4). In this experiment, we have confirmed that our product, TrkB agonist peptide (PG-003), functions as a neurotrophic factor in a similar manner as recombinant BDNF, using SH-SY5Y human neuroblastoma cells (assay performed according to the published methods [5]).

- Cell & Materials :

1) Cell

Designation:	SH-SY5Y (human neuroblastoma cell line)
Source:	ECACC (Cat. No. 94030304)

2) Medium and Cell-culture supplements

Growth medium:	DMEM/F12 (1:1) + 10% Fetal Bovine Serum (FBS) + 1% Penicillin/Streptomycin (P/S)
Differentiation Medium 1:	DMEM/F12 (1:1) + 2.5% FBS + 10 µM Retinoic Acid (RA) + 1% P/S
Differentiation Medium 2:	DMEM/F12 (1:1) + 1% FBS + 10 µM RA + 1% P/S
Differentiation Medium 3:	Neurobasal Medium + 1x B27 + 1% GlutaMax + 10 µM RA + 1% PS + recombinant BDNF (rBDNF) or TrkB agonist peptide (PG-003)
Dissociative Solution:	0.05% Trypsin-EDTA

3) The other reagents

Reagents	Cat. No.	Manufacturer
DMEM (1.0 g/l Glucose) with L-Glutamine and Sodium Pyruvate, liquid (DMEM)	08456-65	Nacalai Tesque
Ham's F-12 Nutrient Mix (F12)	11765054	Thermo Fisher Scientific
Fetal Bovine Serum, certified, United States (FBS; deactivated at 56 ℃ for 30 min)	F7524	Sigma-Aldrich
Penicillin-Streptomycin Mixed Solution (Stabilized) (P/S)	09367-34	Nacalai Tesque
B-27 Supplement (50×), serum free (B27)	17504044	Thermo Fisher Scientific
GlutaMAX Supplement	35050061	Thermo Fisher Scientific
Neurobasal Medium	21103049	Thermo Fisher Scientific

Brain-derived neurotrophic factor (recombinant, human)	B3795	Sigma-Aldrich
Trypsin-EDTA (0.05%), phenol red	25300054	Thermo Fisher Scientific
Poly-D-lysine hydrobromide (Poly-D-lysine)	P7280	Sigma-Aldrich
D-PBS, without Ca and Mg (PBS (-/-))	14190144	Thermo Fisher Scientific
D-PBS (+) Preparation Reagent (Ca, Mg Solution) (100 ×)	02492-94	Nacalai Tesque
Neurite Outgrowth Staining Kit	A15001	Thermo Fisher Scientific

4) Instrument and equipment

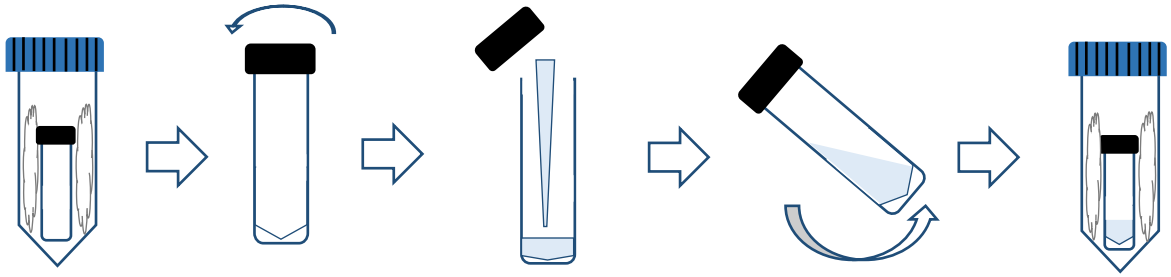
Instrument/equipment	Cat. No.	Manufacturer
Cell/Tissue Culture Flask 75 Filter Cap (T-75 flask)	MS-23250	Sumitomo Bakelite
µ-Plate 24 Well Black ID 14 mm (24 well black plate)	82426	ibidi GmbH
OneCell Counter	BMS-OCC01	Bio Medical Science
PROKEEP protein low binding tube 1.5 mL (1.5 mL protein low binding tube)	PK-15C-500	Watson
Multi-Gas Incubator	APM-30DR	ASTEC
Phase-Contrast Culture Microscope	CKX53	Olympus
Operetta CLS High-Content Analysis System (Operetta CLS)	HH16000000	Perkin Elmer
Harmony High-Content Imaging and Analysis Software (ver. 4.6) (Harmony Software)	HH17000001	Perkin Elmer

- Methods：

1) The following procedures were carried out - under sterile conditions (in a bio- safety-cabinet).
2) One vial of TrkB agonist peptide (PG-003, 10 µg/vial) was centrifuged and the peptide was collected at the bottom. The entire glass vial was wrapped with a soft buffer material in order to prevent them from being destroyed at centrifugation (schematically shown in the following Figure ①).
3) Screw cap of the vial was gently opened (see the following Figure ②), and the whole peptide (10 µg) was dissolved in 350 µL of the Differentiation Medium 3 (basal medium), as shown in the following Figure ③. As a result, TrkB agonist peptide concentration in the solution was 5.55 µM (28.6 µg/mL) (PG-003 Stock solution).
4) Close the cap tightly and dissolve the peptide completely by vortex mixer. At this time, since the peptide content is too low to be visually identified, the walls of the vial were thoroughly rinsed with the added solution (see the following Figure ④).
5) Collect the peptide lysate at the bottom of the vial manually by using the pipet with a tip, or alternatively, re-centrifuge the vial and collect solution at the bottom (see the following Figure ⑤).

6) PG-003 Stock solution (peptide concentration: 28.6 μg/mL = 5.55 μM) was diluted in Differentiation Medium 3 (basal medium) into the solutions with the several concentrations (0, 9.55, 28.65 ng/mL = 0, 1.85, 5.55 nM). Recombinant BDNF was similarly diluted in Differentiation Medium 3 (basal medium) into the solutions with the concentrations (0, 50, 150 ng/mL = 0, 1.85, 5.55 nM) prior to be used in the tests.
7) PG-003 Stock solution was dispensed into the protein-low adsorption tubes (40 μL/tube) and stored at -80℃, except for the first use.
8) At the first medium change, unfrozen solution immediately after stock preparation was used. Later, a fresh stock solution was thawed and used for subsequent media changes.

① Centrifugation ② Cap Opening ③ Solvent addition ④ Rinse walls ⑤ Centrifugation



9) Cell culture and neuronal differentiation: Cells were cultured in the multi-gas incubator (5% CO_2, 3% O_2, 37℃, humidified) according to the - time course below.

Process	Medium	Cell culture substrate	Period or condition
Pre-conditioned	Growth Medium	T-75 flask	Up to 70 ~ 80% confluence
Differentiation 1	Differentiation Medium 1	T-75 flask	3 days
Differentiation 2	Differentiation Medium 2	T-75 flask	3 days
Passage	Differentiation Medium 2	Poly-D-Lysine coated 24 well plate	One day after passage
Differentiation 3	Differentiation Medium 3	Poly-D-Lysine coated 24 well plate	10 days (medium change every 3 days)

10) Neurite outgrowth assay: After Differentiation 3 process, nerve cell membranes and nucleus were stained with the Neurite Outgrowth Staining Kit, according to the manufacture's instruction. After staining, microscopic images were observed and recorded using the Operetta CLS high-content analysis system.
11) Image analysis and statistical analysis: The above image data were analyzed and quantified using

Harmony software to calculate neurite length per cell body. All of the experiments were performed at n = 3, and difference of $p < 0.05$ was judged significant in a Student's T-test (two-tailed) compared with the negative control.

- Results:

The fluorescence microscope images used in the statistical analysis (magnification: 5×, 1 out of 9 graphical fields per well) and unanalyzed microscope images (magnification: 10×, 1 out of 25 images per 1 well) (Figures 1 and 2, respectively).

Similar to the culture condition supplemented with recombinant BDNF, neurite outgrowth was detected to be enhanced in media supplemented with PG -003 (Figures 1 and 2). Furthermore, by image analysis, neurite outgrowth length per cell body was shown to be significantly increased compared to the negative control (at 0 nM), regardless of the supplemented agents at any of the other concentrations (Figure 3).

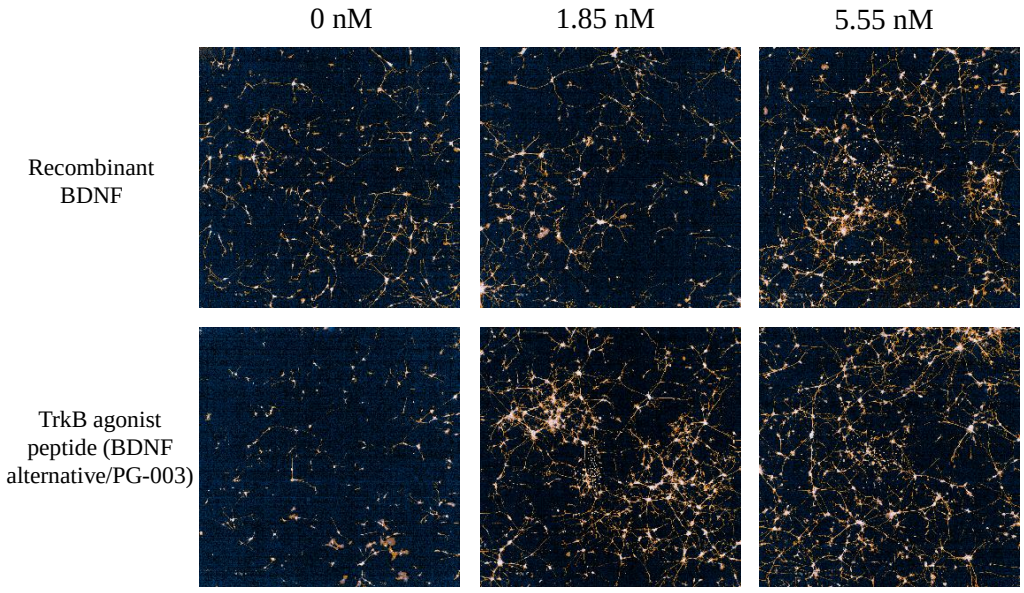


Figure 1: Fluorescence Microscopic Images (5×) of Neurite Outgrowth of Human Neuroblastoma Cell (SH-SY5Y)

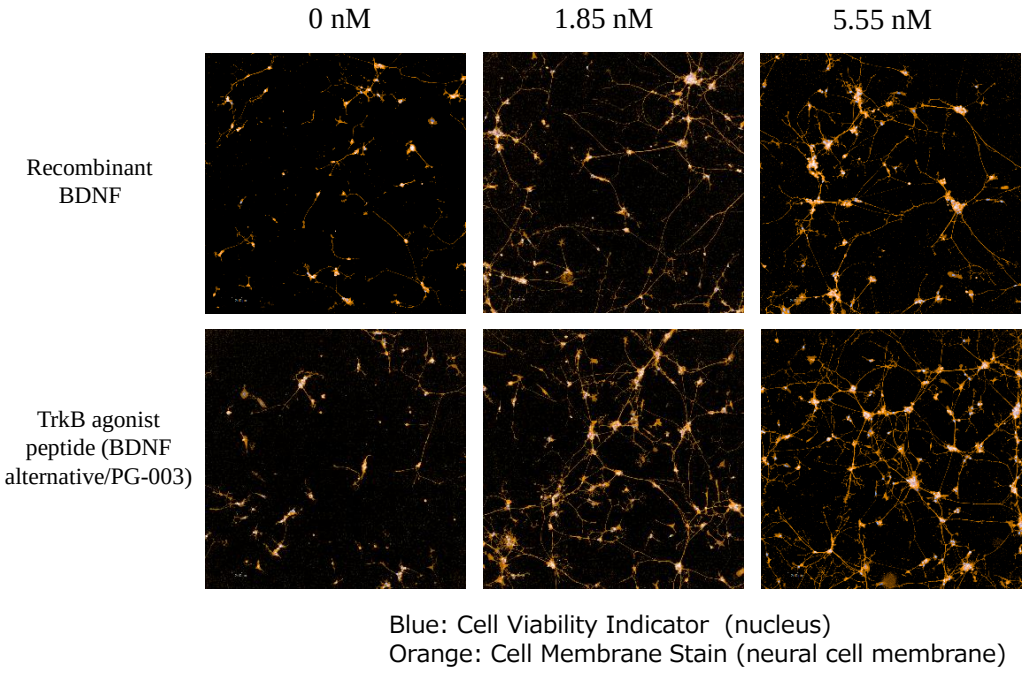


Figure 2: Fluorescence Microscopic Images (10×) of Neurite Outgrowth of Human Neuroblastoma Cell (SH-SY5Y)

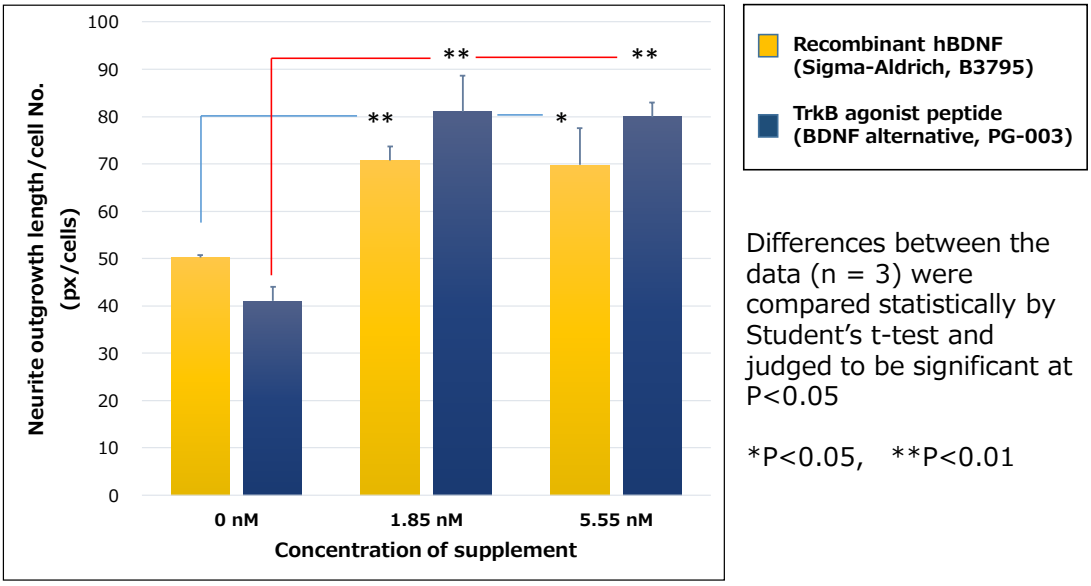


Figure 3: Comparison of Effects of rBDNF and TrkB Agonist Peptide (PG-003) on Neurite Outgrowth in Human Neuroblastoma Cell (SH-SY5Y).

- References:

1) Binder DK & Scharfman HE., *Growth Factors,* 2004; **22**: 123-131.
2) Tapia-Arancibia L, *et al.*, *Brain Res. Rev.,* 2008; **59**: 201-220.
3) Brunoni AR, et al., *Int. J. Neuropsychopharmacol.*, 2008; **11**: 1169-1180.
4) Iwasaki K, *et al., Int. J. Dev. Neurosci.*, 1998; **16**: 135-145.
5) Shipley MM, *et al., J. Vis. Exp.*, 2016; **17**: 53193.

- Notes:
  - Please read the Safety Data Sheet (SDS) prior to use this product.
  - It is highly recommended to use this product immediately after it is dissolved (the above procedures for dispensing and cryopreservation of the products should be regarded as examples). Please contact the manufacturer or your distributor about stability of the dissolved products.
  - This product is for investigational use only.
  - Specifications, content, appearance, etc. of this product may be changed without precaution.
  - Please contact the manufacturer referring to the below contact information or your distributor if you need high volume or other special version of the products.

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