

General Information

Transporters responsible for nutrient uptake have been attracting attention as a target for cancer diagnosis and drug discovery. For example, L-type amino acid transporter 1 (LAT1), which is one of the amino acid (AA) transporters, is specifically expressed in many cancer cells. To assess an AA transporter activity, an assay using radioisotope-labeled AAs has been used as a conventional method. However, this method requires special handling and disposal of radioactive materials.

Amino Acid Uptake Assay Kit allows a simple fluorescence assay for AA transporter activity using Boronophenylalanine (BPA), which is an AA analog used in boron neutron capture therapy for cancer, and the fluorescent probe in this kit. This method can be applied to fluorescence imaging, microplate measurement, and flow cytometry, and is thus useful for evaluating cellular AA uptake activity and screening AA transporter inhibitors.

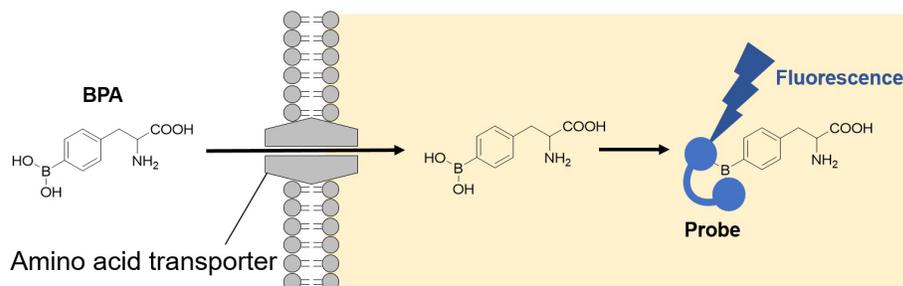


Figure 1. Principle of Amino Acid Uptake Assay Kit

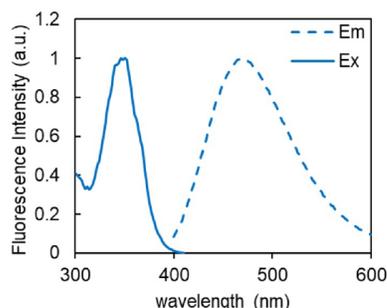


Figure 2. Excitation and emission spectra of Probe-BPA complex

Kit Contents

	20 tests	100 tests
BPA Solution	35 μ l x 1	175 μ l x 1
BPA Dilution Buffer	35 μ l x 1	175 μ l x 1
Probe Solution	15 μ l x 1	75 μ l x 1

Storage Condition

Store at -20°C , and protect from light.

Required Equipment and Materials

- 96 well black microplate
- 20-200 μ l multichannel pipette
- Micropipette
- 15 ml conical tube
- 1.5 ml microtube
- Hanks' Balanced Salt Solution (HBSS) or PBS(+) with 0.1% glucose

Precaution

- Please tap the tube before opening and open it with care. The content may have relocated from the bottom of the tube during the shipping.

Preparation of Solutions

Preparation of BPA uptake solution

Mix BPA Solution with an equal volume of BPA Dilution Buffer. Then, dilute this solution 50-fold with HBSS.

Culture equipment (amount)	Adherent Cells			Suspension Cells	
	6-well (1.5 ml/well)	24-well (0.3 ml/well)	96-well (0.15 ml/well)	35-mm Dish (1.5 ml/well)	1.5 ml microtube (0.5 ml/tube)
HBSS	1500 μ l	300 μ l	150 μ l	1500 μ l	500 μ l
BPA Solution	15 μ l	3 μ l	1.5 μ l	15 μ l	5 μ l
BPA Dilution Buffer	15 μ l	3 μ l	1.5 μ l	15 μ l	5 μ l

Note: PBS(+) with 0.1% glucose can be used instead of HBSS.

Note: Prepare the BPA uptake solution fresh each day and use it immediately after preparation.

Preparation of working solution

Dilute Probe solution 250-fold with HBSS and mix by pipetting and vortex mixing.

	Adherent Cells				Suspension Cells
Culture equipment (amount)	6-well (1.5 ml/well)	24-well (0.3 ml/well)	96-well (0.15 ml/well)	35-mm Dish (1.5 ml/well)	1.5 ml microtube (0.5 ml/tube)
HBSS	1500 μ l	300 μ l	150 μ l	1500 μ l	500 μ l
Probe Solution	6 μ l	1.2 μ l	0.6 μ l	6 μ l	2 μ l

Centrifuge the working solution at 300 x g for 3 min, transfer the supernatant to a new tube.

Note: The undissolved residue of the probe affects observation under a fluorescence microscope and fluorescence measurement with a microplate reader.

Note: Prepare the working solution fresh each day and use it immediately after preparation.

General Protocol

Fluorescence Imaging, Plate reading

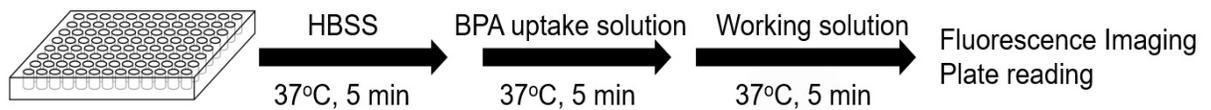


Figure 3. Protocol for fluorescence imaging and plate reading

Note: *1 Amino Acid uptake into the cells may be affected by the temperature. Pre-warm HBSS, BPA uptake solution and working solution in an incubator (37°C).

Note: Use a multichannel pipette to reduce the time difference between each well.

1. Seed cells in a 96-well microplate and culture the cells at 37°C overnight in a 5% CO₂ incubator.
2. Remove the culture medium and wash the cells with pre-warmed HBSS*1 three times.
3. Add 150 μ l of pre-warmed HBSS*1 and incubate at 37°C for 5 minutes in a 5% CO₂ incubator.
4. Remove the supernatant, add 150 μ l of pre-warmed BPA uptake solution*1 or pre-warmed HBSS*1 (blank) and incubate at 37°C for 5 minutes in a 5% CO₂ incubator.
5. Remove the supernatant and wash the cells with pre-warmed HBSS*1 three times.
6. Remove the supernatant, add 150 μ l of pre-warmed working solution*1 and incubate at 37°C for 5 minutes.
7. Observe under a fluorescence microscope or measure the fluorescence intensity with a fluorescence plate reader.
(Fluorescence Imaging: DAPI filter set, Plate reading: Ex/Em = 360/460 nm)

Flow cytometry

(For adherent cells)

1. Seed cells in a 6-well microplate at 2×10^5 cells/well and culture the cells at 37°C overnight in a 5% CO₂ incubator.
2. Remove the supernatant and wash the cells with pre-warmed HBSS*1 three times.
3. Add 1,500 μ l of pre-warmed HBSS*1 and incubate at 37°C for 5 minutes in a 5% CO₂ incubator.
4. Remove the supernatant, add 1,500 μ l of pre-warmed BPA uptake solution*1 or pre-warmed HBSS*1 (blank) and incubate at 37°C for 5 minutes in a 5% CO₂ incubator.
5. Remove the supernatant and wash the cells with pre-warmed HBSS*1 three times.
6. Remove the supernatant, add 1,500 μ l of pre-warmed working solution*1 and incubate at 37°C for 5 minutes.
7. Harvest the cells using a cell scraper.
8. Analyze the cells using a flow cytometer.

(For suspension cells)

1. Dispense the cells to microtubes at 2×10^5 cells/tube.
2. Centrifuge the tubes at 300 x g for 5 minutes, remove the supernatant and add 500 μ l of pre-warmed HBSS*1. Repeat this step twice.
3. Centrifuge the tubes at 300 x g for 5 minutes and remove the supernatant. Add 500 μ l of pre-warmed HBSS*1, suspend by pipetting and incubate for 5 minutes at 37°C.
4. Centrifuge the tubes at 300 x g for 5 minutes and remove the supernatant.
5. Add 500 μ l of pre-warmed BPA uptake solution*1 or pre-warmed HBSS*1 (blank), suspend by pipetting and incubate for 5 minutes at 37°C.
6. Centrifuge the tubes at 300 x g for 5 minutes, remove the supernatant and add 500 μ l of pre-warmed HBSS*1. Repeat this step twice.
7. Centrifuge the tubes at 300 x g for 5 minutes and remove the supernatant. Add 500 μ l of pre-warmed working solution*1, suspend by pipetting and incubate for 5 minutes at 37°C.
8. Analyze the cells using a flow cytometer.

Inhibition of amino acid uptake by BCH (2-Aminobicyclo [2.2.1] heptane-2-carboxylic acid) (HeLa cells)

(Fluorescence imaging and plate reading)

1. HeLa cells (1×10^4 cells/well) in MEM (10% FBS) were seeded in a 96-well black microplate (ib89626, ibidi) and cultured at 37°C overnight in a 5% CO₂ incubator.
2. After removing the supernatant, the cells were washed three times with 150 µl of HBSS (37°C).
3. HBSS containing 0 mmol/l (37°C, sample 1) or 1 mmol/l BCH (37°C, sample 2) were added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
4. After removing the supernatant, BPA uptake solution containing 0 mmol/l BCH (37°C, sample1) , 1 mmol/l BCH (37°C, sample2) or HBSS (37°C, blank) were added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
5. After removing the supernatant, the cells were washed with HBSS (37°C) three times.
6. After removing the supernatant, working solution (37°C) was added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
7. The cells were observed under a fluorescence microscope and the fluorescence intensity was measured with a microplate reader.

(Fluorescence Imaging: DAPI filter set, Plate reading: Ex/Em = 360/460 nm)

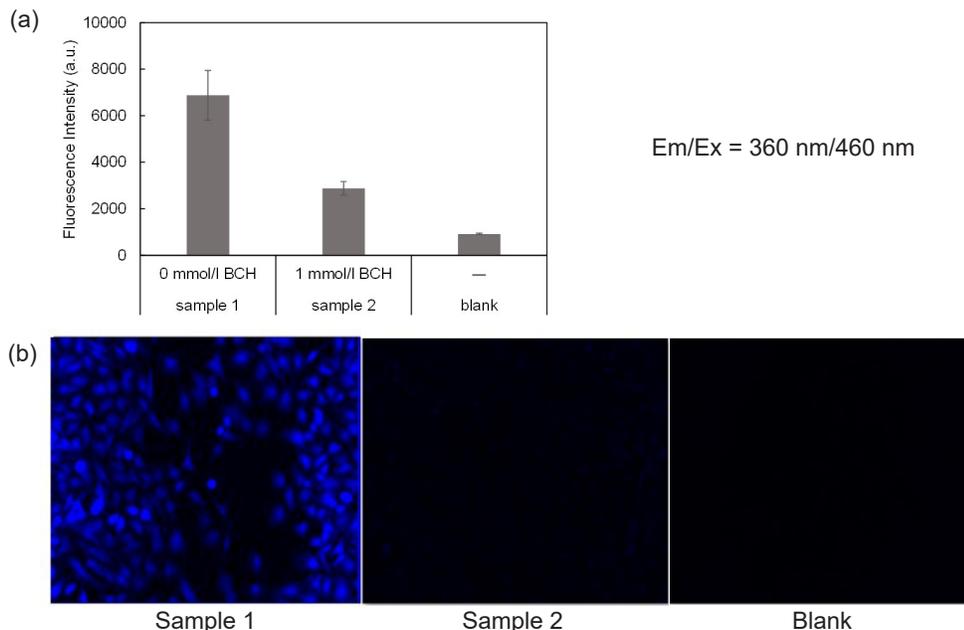


Figure 4. Inhibition of amino acid transporters activity by BCH
(a) microplate reading, (b) fluorescence Imaging

(For flow cytometry)

1. HeLa cells (1.5×10^5 cells/well) in MEM (10% FBS) were seeded in a 6-well microplate (3810-006, AGC techno glass) and cultured at 37°C overnight in a 5% CO₂ incubator.
2. After removing the supernatant, the cells were washed three times with 1,500 µl of HBSS (37°C).
3. HBSS containing 0 mmol/l (sample 1) or 1 mmol/l BCH (sample 2) were added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
4. After removing the supernatant, BPA uptake solution (37°C) including 0 mmol/l BCH (sample1), 1 mmol/l BCH (sample2) or HBSS (37°C, blank) were added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
5. After removing the supernatant, the cells were washed with HBSS (37°C) three times.
6. After removing the supernatant, working solution (37°C) was added and the cells were incubated at 37°C for 5 minutes in a 5% CO₂ incubator.
7. The cells were harvested using a cell scraper and analyzed with a flow cytometer (LSR Fortessa X-20, BD).
BV421 filter set (Ex:405 nm, Em:450/40 nm)

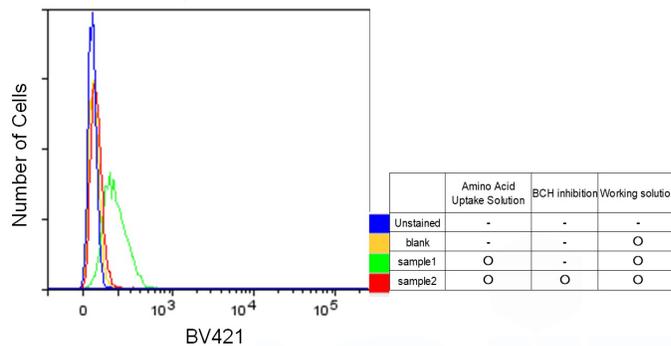


Figure 5. Inhibition of amino acid transporters activity by BCH (Flow cytometry)

This product was developed by the kind support of Ph.D. Mitsunori Kirihata at Research Center of Boron Neutron Capture Therapy, Research Organization for the 21st Century, Osaka Prefecture University.

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UP04 : Amino Acid Uptake Assay Kit