Version 1c Last updated 27 June 2022

ab228552 Intracellular pH Assay Kit

For the measurement of pH in adherent and non-adherent cells

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Intracellular pH Assay Kit (ab228552) utilizes a proprietary fluorescent indicator for measuring relative intracellular pH changes. It is a homogeneous, kinetic, live-cell fluorescent assay that utilizes either a standard procedure or acid-load procedure. The standard protocol is designed for measuring the therapeutic targets of interest with a decrease in intracellular pH upon treatment. The 'Acid-Load' procedure is designed to measure the increase of intracellular pH associated with changes in cellular metabolism due to GPCR activation or growth factor activity. With the 'Acid-Load' procedure ammonium chloride solution is added after the fluorescent pH dye is loaded into cells in a minimum volume. This 'acid-loading' step is followed by the addition of agonist in a relatively large volume (~4X) of buffer. The sudden volume change initiates an efflux of ammonia (NH_3) from the cells causing a rapid decrease in intracellular pH, and thus a decrease in fluorescence signal. The effect of agonist on the subsequent recovery of intracellular pH is measured by the relative fluorescence signal increase.

Prepare cells in growth medium. Remove medium for Acid Load procedure.

⇩

Add BCFL, AM dye-loading solution. Incubate at 37°C for 1 hour.

⇩

For Acid Load procedure add 5 L/well of 220 mM NH_4Cl and incubate at RT for 15 minutes

⇩

Read Fluorescence at Ex/Em= 490/535 nm with 200 µL/well compound addition (or 505/535 and 430/535 nm for ratio)

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Reconstituted components are stable for 1 month.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
BCFL, AM	1 vial	-20°C	-20°C
10X Pluronic F127 Plus	10 mL	-20°C	-20°C
HHBS (Hanks' buffer with 20 mM Hepes)	100 mL	-20°C	-20°C
50 mM probenecid	10 mL	RT	RT

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em 490/535 nm or 505/535 and 430/535 for ratio measurements
- 96 or 384-well (Standard Load only) plates
- DMSO
- NH_4Cl

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

- Briefly centrifuge small vials at low speed prior to opening.
- Thaw all components to room temperature (18-25°C) prior to use.

5.1 BCFL, AM for Standard Cell Load (one plate)

1. Make BCFL, AM stock solution by adding 200 µL DMSO into BCFL, AM and mixing them well.
   Δ Note: 20 µL of reconstituted BCFL, AM is enough for 1 plate. Unused BCFL, AM can be aliquoted and stored at ≤-20°C for one month if the tubes are sealed tightly, avoided light and repeated freeze-thaw cycles.
2. Make 1X assay buffer by adding 1 mL of 10X Pluronic F127 Plus into 9 mL of HHBS (Hanks' buffer with 20 mM Hepes), mix them well.
   Δ Note: Note: For cells that require probenecid for loading (e.g. CHO cells), dilute 50 mM probenecid at concentration of 1 to 5 mM (prefer 5 mM for CHO cells).
3. Make BCFL, AM dye-loading solution for one cell plate by adding 20 µL of DMSO reconstituted BCFL, AM (from Step 5.1.1) into 10 mL of 1X assay buffer (from Step 5.1.2), mixing them well. This working solution is stable for at least 2 hours at room temperature.

5.2 BCFL, AM for Acid Load (one 96 well plate)

1. Make BCFL, AM stock solution by adding 200 µL DMSO into BCFL, AM and mixing them well.
   Δ Note: 10 µL of reconstituted BCFL, AM is enough for 1 plate. Unused BCFL, AM can be aliquoted and stored at ≤-20°C for one month if the tubes are sealed tightly, avoided light and repeated freeze-thaw cycles.
2. Make 1X assay buffer by adding 1 mL of 10X Pluronic F127 Plus into 4 mL of HHBS (Hanks' buffer with 20 mM Hepes), mix them well.
   Δ Note: Note: For cells that require probenecid for loading (e.g. CHO cells), dilute 50 probenecid at concentration of 0.5 to 2.5 mM (prefer 5 mM for CHO cells).
3. Make BCFL, AM dye-loading solution for one cell plate by adding 10 µL of DMSO reconstituted BCFL, AM (from Step 5.2.1) into 5 mL of 1X assay buffer (from Step 5.2.2), mixing them well.

This working solution is stable for at least 2 hours at room temperature.

6. Sample Preparation

6.1 Adherent cells:

1. Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/100µL for 96-well or 10,000 to 20,000 cells/well/25µL for 384-well plates (for Standard Cell Load only).

6.2 Non-adherent cells:

1. Centrifuge the cells from the culture medium and then suspend the cell pellets in growth medium at 125,000 to 250,000 cells/well/100µL for 96-well or 30,000 to 60,000 cells/well/25µL for 384-well (for Standard Cell Load only) poly-D lysine plates.
2. Centrifuge the plates at 800 rpm for 2 minutes with break off prior to the experiments.
   Δ **Note:** Each cell line should be evaluated on an individual basis to determine the optimal cell density.

7. Assay Procedure

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

7.1 Standard Cell Load

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) BCFL, AM dye-loading solution into the cell plate (from Step 5.1.3).
   Δ Note: It is important to replace the growth medium with HHBS buffer (100 µL/well for 96-well plate or 25 µL/well for 384-well plate before dye-loading) if your compounds interfere with the serum.
2. Incubate the dye-loading plate at cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.
   Δ Note: If the assay requires 37°C, perform the experiment immediately without further room temperature incubation.
3. Prepare the compound plates by using HHBS or your desired buffer.
4. Run the pH assay by monitoring the fluorescence at Ex/Em = 490/535 nm (cut off at 515 nm) or 505/535 nm and 430/535 nm (cut off at 515 nm) for ratio measurements. The compound addition is 50 µL/well (96-well plate) or 25 µL/well (384-well plate).
   Δ Note: The assay should be complete within 3 to 5 min after compound addition, however a minimum of 8 min data collection are recommended for during assay development.

7.2 Acid Load:

1. Remove the growth medium from the cell plate.
2. Add 50 µL/well/96-well plate BCFL, AM dye-loading solution into the cell plate (from Step 5.2.3).
3. Incubate the dye-loading plate at cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.
   Δ Note: If the assay requires 37°C, perform the experiment immediately without further room temperature incubation.
4. Add 5 µL of 220 mM NH_4Cl and then centrifuge the plates for 5 seconds, and incubate 15 minutes at room temperature.

Δ Note: NH_4Cl solution should be prepared freshly in HHBS.

5. Prepare the compound plates by using HHBS or your desired buffer.
6. Run the pH assay by monitoring the fluorescence at Ex/Em = 490/535 nm (cut off at 515 nm) or 505/535 nm and 430/535 nm (cut off at 515 nm) for ratio measurements. The compound addition is 200 µL/well/96-well plate.
   Δ Note: The assay should be complete within 3 to 5 min after compound addition, however a minimum of 8 min data collection are recommended for during assay development.

8. FAQs / Troubleshooting

General troubleshooting points can be found at www.abcam.com/assaykitguidelines.

9. Typical Data

Data provided **for demonstration purposes only.**

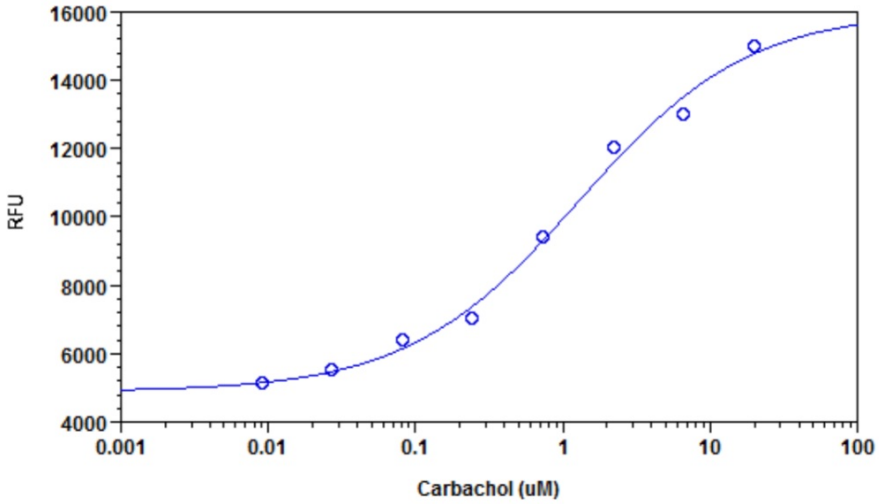

Figure 1. Carbachol dose response in CHO-M1 cells. CHO-M1cells were seeded overnight in 60,000 cells per 100 µL per well in a 96-well black wall/clear bottom Costar plate. The growth medium was replaced with 50 µL/well of BCFL, AM dyeloading solution for 37°C for 1 hour, follow by 15 minutes incubation with 5 µL/well of 220 mM NH_4Cl. Carbachol (200µL/well) was added by FlexStation (Molecular Devices) to achieve the final indicated concentrations. The fluorescent signal was generated using Ex/Em = 490/535 nm (cut off at 525 nm).

10. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)