# Cell Meter™ Fluorimetric Intracellular pH Assay Kit

Catalog number: 21180
Unit size: 1000 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: RatioWorks™ BCFL, AM | Freeze (< -15 °C), Minimize light exposure | 1 vial |
| Component B: 10X Pluronic F127 Plus | Freeze (< -15 °C), Minimize light exposure | 1 bottle (10 mL) |
| Component C: HHBS (Hanks' buffer with 20 mM Hepes) | Freeze (< -15 °C), Minimize light exposure | 1 bottle (100 mL) |
| Component D: 50 mM ReadiUse™ probenecid | Freeze (< -15 °C), Minimize light exposure | 1 bottle (10 mL) |

## OVERVIEW

Intracellular pH change are implicated in diverse physiological and pathological processes, including cell proliferation, apoptosis, fertilization, malignancy, multidrug resistance, ion transport, lysosomal storage disorders and Alzheimer's disease. The Cell Meter™ Fluorimetric Intracellular pH Assay Kit utilizes AAT Bioquest's proprietary fluorescent indicator for measuring the 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 (NH3) 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.

## AT A GLANCE

**Protocol Summary**
**for Standard Cell Load (One Plate)**

1. Prepare cells in growth medium
2. Add equal volume of RatioWorks™ BCFL, AM dye-working solution
3. Incubate at 37 °C for 1 hour
4. Read Fluorescence at Ex/Em= 490/535 nm (Cutoff = 515 nm) with 50 µL/well compound addition (or 500/530 nm and 440/530 nm for ratio)

**Protocol Summary**
**for Acid-Load (One 96-well Plate)**

1. Prepare cells in growth medium
2. Remove the growth medium
3. Add RatioWorks™ BCFL, AM dye-working solution (50 µL/well/96-well plate)
4. Incubate at 37°C for 1 hour
5. Add 220 mM $NH_4Cl$ (5 µL/well)
6. Incubate at RT for 15 minutes
7. Read Fluorescence at Ex/Em= 490/535 nm (Cutoff = 515 nm) with 200 µL/well compound addition (or 500/530 nm and 440/530 nm for ratio)

**Important** Thaw all the kit components at room temperature before starting the experiment.

## KEY PARAMETERS

**Fluorescence microplate reader**

| | |
|---|---|
| Excitation | 490 nm |
| Emission | 535 nm |
| Cutoff | 515 nm |
| Recommended plate | Black well/Clear bottom |

**Other instruments**

FDSS, FLIPR, ViewLux, NOVOStar, ArrayScan, FlexStation, IN Cell Analyzer

## CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

## PREPARATION OF STOCK SOLUTIONS

*Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.*

**RatioWorks™ BCFL, AM stock solution**
Add 200 µL DMSO into the vial of RatioWorks™ BCFL, AM (Component A) and mix well to make RatioWorks™ BCFL, AM stock solution.

**Note** 20 µL of reconstituted RatioWorks™ BCFL, AM stock solution is enough for 1 plate of Standard Cell Load. 10 µL of reconstituted RatioWorks™ BCFL, AM stock solution is enough for 1 plate of Acid Load. RatioWorks™ BCFL, AM stock solution can be stored at ≤ -20 ° C for one month if the tubes are sealed tightly. Protect from light.

## PREPARATION OF WORKING SOLUTION

**1. For Standard Cell Load (One Plate)**

1. Add 1 mL of 10X Pluronic F127 Plus (Component B) into 9 mL of HHBS (Component C) and mix well to make 1X assay buffer.

   **Note** For cells that require probenecid for loading (e.g. CHO cells), dilute 50 mM ReadiUse™ Probenecid (Component D) at concentration of 1 to 5 mM (prefer 5 mM for CHO cells).
2. Add 20 µL of reconstituted RatioWorks™ BCFL, AM stock solution into 10 mL of 1X assay buffer and mix well to make RatioWorks™ BCFL, AM dye-working solution. This RatioWorks™ BCFL, AM dye-working solution is stable for at least 2 hours at room temperature.

**2. For Acid-Load (One 96-well Plate)**

1. Add 1 mL of 10X Pluronic F127 Plus (Component B) into 4 mL of HHBS (Component C) and mix well to make 1X assay buffer.

   **Note** For cells that require probenecid for loading (e.g. CHO cells), dilute 50 mM ReadiUse™ Probenecid (Component D) at concentration of 0.5 to 2.5 mM (prefer 2.5 mM for CHO cells).
2. Add 10 µL of reconstituted RatioWorks™ BCFL, AM stock solution into 5 mL of 1X assay buffer and mix well to make RatioWorks™ BCFL, AM dye-working solution. This RatioWorks™ BCFL, AM dye-working solution is stable for at least 2 hours at room temperature.

## SAMPLE EXPERIMENTAL PROTOCOL

**Run pH Assay for Standard Cell Load**

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) RatioWorks™ BCFL, AM dye-working solution into the cell plate.

   **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 in cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes.

   **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 (Cutoff = 515 nm) or 500/530 nm and 440/530 nm (Cutoff = 515 nm) for ratio measurements. The compound addition is 50 µL/well (96-well plate) or 25 µL/well (384-well plate).

   **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.

**Run pH Assay for Acid-Load**

1. Remove the growth medium from the cell plate.

2. Add 50 µL/well/96-well plate RatioWorks™ BCFL, AM dye-working solution into the cell plate.

3. Incubate the dye-loading plate in cell incubator for 30 minutes, and then incubate the plate at room temperature for another 30 minutes. *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 centrifuge the plates for 5 seconds. Incubate 15 minutes at room temperature.

   **Note** $NH_4Cl$ solution should be prepared freshly in HHBS (Component C).

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 (Cutoff = 515 nm) or 500/530 nm and 440/530 nm (Cutoff = 515 nm) for ratio measurements. The compound addition is 200 µL/well/96-well plate.

   **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.

## EXAMPLE DATA ANALYSIS AND FIGURES

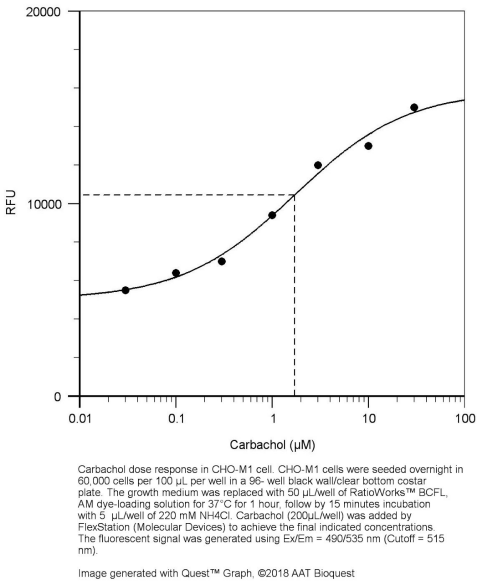


**Figure 1.** Carbachol dose response in CHO-M1 cell. CHO-M1 cells were seeded overnight in 60,000 cells per 100 µL per well in a 96- well black wall/clear bottom costar plate. The growth medium was replaced with 50 µL/well of RatioWorks™ BCFL, AM dye-loading solution for 37°C for 1 hour, follow by 15 minutes incubation with 5 µL/well of 220 mM NH4Cl. 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 (Cutoff = 515 nm).

## DISCLAIMER

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