TECHNICAL MANUAL

Intracellular Acidification Rate Fluorometric Assay Kit

- **SKU CODE:** MAES0316
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure the intracellular acidification rate in cell samples.

2. Detection principle

Intracellular acidification rate refers to the degree of pH change in the intracellular environment, which is an important parameter for studying cell function. Maintaining a constant intracellular pH level is essential for normal cellular metabolism and function. Most physiological and biochemical reactions within the cell, such as cell growth, calcium regulation, enzyme activity, receptor-mediated signal transduction, ionic actions, endocytosis, and cell adhesion, depend on proper pH regulation. Therefore, there is a close relationship between intracellular pH regulation and metabolism, and accurate detection of intracellular pH fluctuations is an important method for understanding intracellular metabolism. Additionally, studying the interaction between intracellular pH regulation and metabolism helps provide better understanding of biochemical processes in cells and offers new insights and methods for treating related diseases.

The probe used in this assay kit has dual excitation wavelength characteristics. The probe exhibits maximum absorption at 490 nm and an isosbestic point at 440 nm. By calculating the ratio of the two excitation wavelengths, fluctuations in intracellular pH can be detected.

3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Saline Solution	12 mL x 1 vial	24 mL x 1 vial	-20 °C, 12 months
Reagent 2	Probe	Liquid x 1 vial	Liquid x 2 vials	-20 °C, 12 months, protect from light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex: 490 nm & 440 nm; Em: 535 nm), Incubator (37 °C)

Reagents:

DMSO

5. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of high concentration probe stock solution:** Dilute one vial of probe with 108 µL of DMSO and mix well to dissolve. Store aliquots at -20 °C for up to 1 month.
3. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the number of test wells. For example, to prepare 500 µL of working solution, thoroughly mix 495 µL of saline solution with 5 µL of high concentration probe stock solution. The working solution should be prepared fresh (the probe is unstable in saline solution, and the working solution is effective within 1 hour after preparation; it is recommended to prepare the working solution after cell pretreatment).

6. Operating steps

1. **Sample preparation:** Suspension cells: Perform cell culture according to experimental design, ensuring cells are healthy and not overgrown. Centrifuge at 500 xg for 5 min at 4 °C and discard the supernatant. Adherent cells: Perform cell culture according to experimental design, ensuring cells are healthy and not overgrown. Remove cell culture medium, digest cells with trypsin, add serum-containing medium to stop digestion, wash and collect cells with normal saline (0.9% NaCl), centrifuge at 500 xg at 4 °C for 5 min, and discard the supernatant.
2. Resuspend cells with working solution at a recommended cell density of 2.5 x 10^6 cells/mL.
3. Blank well: Add 200 µL working solution to the wells. Sample well: Add 200 µL cell suspension to the wells.
4. Mix thoroughly for 3 seconds using the fluorescence microplate reader. Measure fluorescence intensity at excitation wavelength 490 nm and emission wavelength 535 nm as F_1(490). Measure fluorescence intensity at excitation wavelength 440 nm and emission wavelength 535 nm as F_1(440).

5. Incubate at 37 °C for 30 minutes protected from light. The incubation time can be adjusted between 10 and 60 minutes, depending on cell type and probe concentration.
6. Mix thoroughly for 3 seconds using the fluorescence microplate reader. Measure fluorescence intensity at excitation wavelength 490 nm and emission wavelength 535 nm as F_2(490). Measure fluorescence intensity at excitation wavelength 440 nm and emission wavelength 535 nm as F_2(440).

7. Calculation

The sample:

Intracellular acidification rate (%) = [ΔF_1(490)/ΔF_1(440) - ΔF_2(490)/ΔF_2(440)] / [ΔF_1(490)/ΔF_1(440)] × 100%

[Note]

ΔF_1(490): The absolute fluorescence value at excitation wavelength 490 nm before incubation, $F_{1sample}(490) - F_{1blank}(490)$.

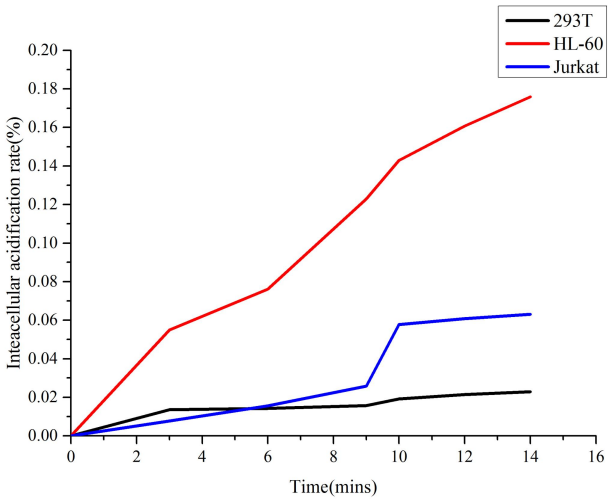
ΔF_1(440): The absolute fluorescence value at excitation wavelength 440 nm before incubation, $F_{1sample}(440) - F_{1blank}(440)$.

ΔF_2(490): The absolute fluorescence value at excitation wavelength 490 nm after incubation, $F_{2sample}(490) - F_{2blank}(490)$.

ΔF_2(440): The absolute fluorescence value at excitation wavelength 440 nm after incubation, $F_{2sample}(440) - F_{2blank}(440)$.

8. Appendix I Performance Characteristics

Intracellular acidification rate of 293T, HL-60, Jurkat (same cell volume)



9. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.