Extracellular Acidification Rate (ECAR) Assay Kit

Catalog No.: abx294412

Size: 96 tests

Storage: Store all components at -20°C in the dark.

Application: For the detection and quantification of Extracellular Acidification Rate (ECAR) in cell samples.

Principle of the Assay

Abbexa's Extracellular Acidification Rate (ECAR) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ECAR in cultured cells. During anerobic respiration, cells release acidic metabolites into the extracellular environment, causing a decrease in pH. A pH-sensitive fluorescent probe shows reduction in intensity in response to decreasing pH, with an excitation wavelength of 490 nm, and an emission wavelength of 535 nm. The intensity of the fluorescence is inversely proportional to the degree of extracellular acidification, which allows ECAR to be calculated.

Kit components

1. Black-Walled, Clear-Bottom 96-well Microplate: 2
2. Probe Reagent: 2 vials
3. Saline Solution: 60 ml
4. Plate sealer: 4

Materials required but not provided

1. Fluorescence microplate reader (Excitation: 490 nm, Emission: 535 nm)
2. DMSO
3. Pipette and pipette tips
4. Centrifuge and centrifuge tubes
5. Vortex mixer
6. Incubator

For Reference Only

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Probe Stock Solution:** Dilute 1 vial of Probe Reagent with 270 µl of DMSO and mix thoroughly. Unused Probe Stock Solution can be stored at -20°C for up to 2 weeks.
- **Probe Working Solution**: Prepare enough Probe Working Solution for the wells to be tested. Mix Probe Stock Solution with Saline Solution in a ratio of 1 : 19. This solution should be prepared just before use.

	1 well	10 wells	50 wells	100 wells
Saline Solution (µl)	95	950	4750	9500
Probe Stock Solution (µl)	5	50	250	500

Note:

- Allow all reagents to equilibrate to room temperature before use.
- The Saline Solution contains 20 mM glucose. If this interferes with the experimental design, HBSS buffer or serum-free medium may be used in place of the Saline Solution.
- Preheat the Saline Solution and Probe Working Solution to 37°C before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

Culture cells according to the experimental design, ensuring that the cells remain healthy and do not become over-confluent.

- **Suspension Cells:** Seed cells into a Black-Walled, Clear-Bottom 96-well Microplate by adding 100 µl of cell suspension to each well. The recommended cell density is 2×10^5 cells/ml (for the blank well, add 100 µl of Saline Solution). Collect the cells by centrifugation at 500 × g for 5 minutes at 4°C. Carefully remove the supernatant and retain the cell pellet. Resuspend 2×10^5 cells in 1 ml of Saline Solution. Add any appropriate stimulatory compounds or drugs according to the experimental design.
- **Adherent Cells:** Seed cells into a Black-Walled, Clear-Bottom 96-well Microplate by adding 100 µl of cell suspension to each well. The recommended cell density is 2×10^5 cells/ml (for the blank well, add 100 µl of Saline Solution). Incubate the plate overnight in a humidified incubator at 37°C with 5% CO_2. Carefully remove the culture medium and add 100 µl of saline solution to each well. Add any appropriate stimulatory compounds or drugs according to the experimental design.

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Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

B. Assay Procedure

Pre-set the fluorescence microplate reader to 37°C prior to measurement.

1. Mark positions on the 96-well microplate for each blank and sample. *It is strongly recommended to test all samples in duplicate*.
2. Cover with a plate sealer, then incubate at 37°C for 30 minutes in the dark.
3. Unseal the plate, then add 100 µl of Probe Working Solution to each well.
4. Immediately measure the fluorescence kinetics in each well using a fluorescence microplate reader with an excitation wavelength of 490 nm and an emission wavelength of 535 nm. Record the fluorescence every 2–3 minutes for a total of 100–120 minutes.

C. Calculation of Results

1. Plot fluorescence intensity (F) against time (min), with fluorescence intensity on the y-axis and time on the x-axis. Select the linear portion of the resulting curve and perform linear regression to obtain a straight-line equation, expressed as $y = ax + b$.

2. For each sample, calculate the difference in absorbance between the two measurements: $\Delta F = F_2 - F_1$.

$$\mathbf{ECAR} = \frac{\mathbf{\Delta F_{Sample}} - \mathbf{\Delta F_{Blank}}}{\mathbf{T_2} - \mathbf{T_1}}$$

where:

ΔF_{Sample}	ΔF value of the sample.
ΔF_{Blank}	ΔF value of the blank
T_1	The starting time point of the selected linear region
T_2	The ending time point of the selected linear region

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Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.