EZ-TITER
Cell Proliferation Reagent
Cat# EZ-TITER

INSTRUCTION MANUAL ZBM0070.00

STORAGE CONDITIONS

- **EZ-TITER Reagent:** 4°C

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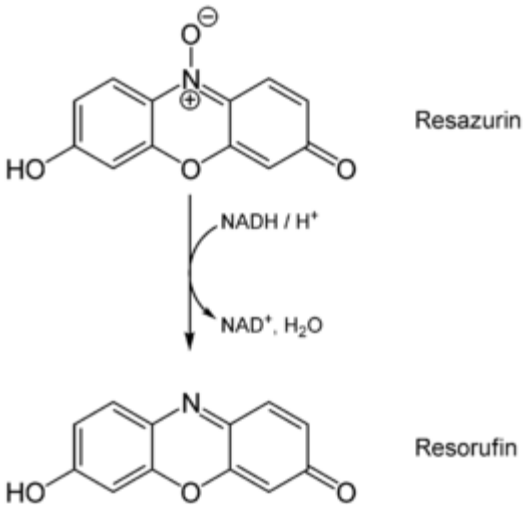
I. INTRODUCTION

Cell proliferation and viability measurements are important tools for research. The EZ-Titer assay is a fluorometric method for estimating the number of viable cells present in multiwell plates. It uses the indicator dye resazurin to measure metabolic capacity of cells-an indicator of viability. Cells that are metabolizing maintain a reducing environment within their cytosol. Non-viable cells rapidly lose metabolic activity and do not reduce the resazurin, and subsequently do not generate a fluorescent signal.



II. LIST OF COMPONENTS

Premixed EZ-Titer is a dark blue solution that is ready to use out of the bottle. If precipitates or turbidity is observed after thawing, place in 37°C water bath to dissolve any precipitates. Once EZ-Titer is thawed, store at 4°C in light protected bottle for several weeks. If longer storage is anticipated, store in aliquots at -20°C protected from light. Avoid freeze-thaw cycles.

The EZ-Titer reagent contains 20 mL. This is enough reagent for 2,000 reactions at 10ul/reaction in a 96-well plate.

III. Additional Materials Required

The following materials are required but not supplied.

Incubator

Shaker

Multiwell fluorescent plate reader for wavelength between 480-600 nm. (560(20)Ex/590(10)Em)

Multichannel pipettors

Single channel pipettors

Centrifuge tubes

Sterile pipet tips

96-well tissue culture plates

Microscope

Trypan blue

Hemacytometer.

IV. Proliferation Assay Protocol

Protocol: Measuring Cell Proliferation

Culture cells in flat bottomed 96-well plates in a final volume of 100 µl/well culture medium in a humidified atmosphere (e.g. 37˚C, 5% CO2). Reserve one well as a background control (culture medium without cells).

Note: The incubation period and cell density of the culture depend on the particular experimental conditions and on the cell line used. For most experimental setups, a cell density between 1000 and 5 x 10^4 cells/well and an incubation time of 24 to 96 hr is appropriate.

Add 10 µl of EZ-Titer Cell Proliferation Reagent to each well (1:10 final dilution).

Note: EZ-Titer Cell Proliferation Reagent should be used at a final dilution of 1:10. If cells are cultured in 200 µl culture medium, add 20 µl EZ-Titer Cell Proliferation Reagent per well.

Incubate the plate for 0.5 to 4 hr at 37˚C in a humidified atmosphere maintained at 5% CO2.

Shake thoroughly for 1 minute on a shaker.

Measure the fluorescence at **(560(20)Ex/590(10)Em)**, using a multiwell plate reader. The reference wavelength should be greater than 600 nm.

Note: The absorbance level of the background control well (containing culture medium plus EZ-Titer Cell Proliferation Reagent, without cells) will depend on the culture medium, incubation time, and exposure to light. Typical background absorbance after 2 hr is between 0.1–0.2 absorbance units.

Protocol: Optimizing Incubation Period

The appropriate incubation time after the addition of EZ-Titer Proliferation Reagent depends on the individual experimental setup (e.g. cell type and cell density used). Therefore, we recommend that you perform a preliminary experiment where the absorbance is measured repeatedly at several time points (i.e., 0.5, 1, 2 and 4 hr) after the addition of the EZ-Titer Cell Proliferation Reagent. If higher sensitivity is required, incubate cells in EZ-Titer Cell Proliferation Reagent for longer periods of time.

V. Cytoxicity Assay Protocol _____

Another application of the EZ-Titer Cell Proliferation Assay is to assess viability in response to cytotoxic and cytostatic compounds. In this example, the assay is used to measure the cytotoxic effect of human tumor necrosis factor (TNF-a) on responsive cell lines.

Additional Reagents:

RPMI 1640 culture medium, containing 10% heat-inactivated FCS, 2 mM L-glutamine, actinomycin C1(1µg/ml) and 1 µg/ml (optionally, add penicillin/streptomycin or gentamicin)

Human TNF-a (10 mg/ml), sterile

Responsive cell line

Protocol Application:

1. Preincubate cells at a density of 1 x 10^6 cells/ml in culture medium with actinomycin C1(1 µg/ml) for 3 hr at 37˚C and 5% CO2.
2. Seed cells at a density of 5 x 10^4 cells/well in 100 µl culture medium containing actinomycin C1 (1 µg/ml) and various amounts of TNF-a (final concentrations of 0.001–0.5 ng/ml) in flat bottom 96-well tissue culture plates. Reserve one well without cells for a background control (culture medium only).
3. Incubate the plate for 24 hr at 37˚C in a humidified atmosphere maintained at 5% CO2.
4. Add 10 µl of EZ-Titer Cell Proliferation Reagent to each well.
5. Incubate for 4 hr at 37˚C and 5% CO2.
6. Shake thoroughly for 1 minute on a shaker.
7. Measure the fluorescence at **(560(20)Ex/590(10)Em)**, using a multiwell plate reader. The reference wavelength should be greater than 600 nm.

APPENDIX A: PLATE LAYOUT _____

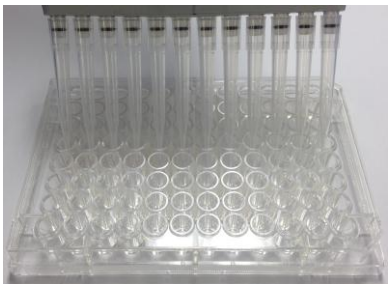
H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

APPENDIX B: PROCEDURE FLOWCHART

A flow diagram summarizing the EZ-Titer. The procedure involves adding EZ-Titer directly to assay, incubation, and recording data with a fluorescence plate reader.

DAY OF ASSAY

Add EZ-Titer reagent to assay.



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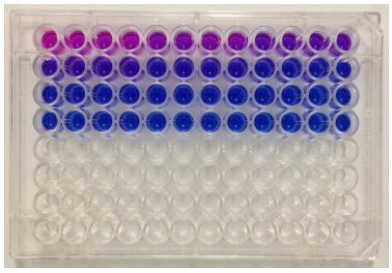
Incubate 1 to 4 hours at 37°C.



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Measure the optical density of each well at **(560(20)Ex/590(10)Em),** using a spectrophotometer plate reader.