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siehe unsere Liefer- und Versandbedingungen

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Resazurin Cell Viability Assay Kit

Item No. 702540

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400633	Resazurin Cell Viability Reagent	2 vials/12 ml	-20°C
601772	Staurosporine Apoptosis Inducer	1 vial/100 µl	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring fluorescence with excitation and emission wavelengths of 560 and 590 nm, respectively
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. Black tissue culture treated plate (96-well recommended)

INTRODUCTION

About This Assay

Cayman's Resazurin Cell Viability Assay Kit provides a non-toxic and easy-to-use method for analyzing cell viability. Resazurin is a redox-sensitive fluorophore that can be irreversibly reduced by the mitochondrial oxidoreductase enzymes in metabolically active cells to produce resorufin.[1-3] Resorufin's fluorescence can be easily quantified using a fluorescence plate reader at excitation and emission wavelengths of 560 and 590 nm, respectively. Non-viable cells lack the ability to reduce resazurin to resorufin, and therefore cannot generate a fluorescent signal. The fluorescent signal generated in the assay is proportional to the number of viable cells present.[1-3]

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Resazurin Cell Viability Reagent - (Item No. 400633)**

   Each vial contains 12 ml of Resazurin Cell Viability Reagent, pH 7.4. Thaw at room temperature. Once thawed, the Resazurin Cell Viability Reagent will be stable for at least three months when stored at 4°C. If kept frozen at -20°C, the reagent will remain stable for at least one year. The reagent can be re-frozen and thawed up to three times without affecting assay performance.

2. **Staurosporine Apoptosis Inducer - (Item No. 601772)**

   This vial contains 100 µl of 1 mM staurosporine in DMSO. The reagent is ready to use as supplied. If not all of the Staurosporine Apoptosis Inducer will be used at one time, aliquot and store at -20°C. Avoid multiple freeze-thaw cycles.

ASSAY PREPARATION

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Performing the Assay

1. Seed cells in a black tissue culture treated, 96-well plate at a density of 10,000-50,000 cells per well in 100 µl of culture medium with or without compounds to be tested. Optimal cell seeding density will be dependent on cell type and duration of the experimental protocol. Culture cells in a CO_2 incubator at 37°C for 24-48 hours. *NOTE: It is recommended to include two wells with 100 µl of culture medium without cells for use as a background control and two wells of cells without compounds for use as an untreated control.*
2. Negative Control (Optional): Add 2 µl of the Staurosporine Apoptosis Inducer per 100 µl of culture media. *NOTE: Cytotoxicity of the Staurosporine Apoptosis Inducer will vary by cell type but should be added at least 3 hours before addition of the Resazurin Cell Viability Reagent.*
3. Add 10 µl of the Resazurin Cell Viability Reagent per 100 µl of culture media.
4. Incubate the cells for 1-5 hours in a CO_2 incubator at 37°C.
5. Read fluorescence using excitation and emission wavelengths of 560 and 590 nm, respectively. Fluorescence can be read at multiple time points to optimize incubation time for different cell types and densities.

Performance Characteristics

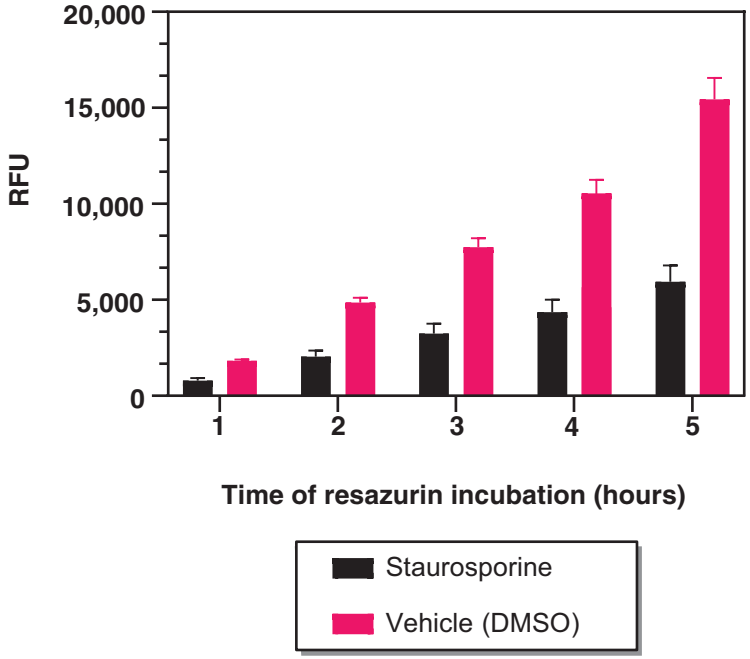

Figure 1. Staurosprine treatment of A549 cells. Cells were seeded at 10,000 cells per well in 100 µl of medium and allowed to attach overnight. Cells were treated with Staurosporine Apoptosis Inducer (20 µM) or vehicle (DMSO, 2%) for three hours before adding 10 µl of Resazurin Cell Viability Reagent. The fluorescence intensity was measured at different time intervals.

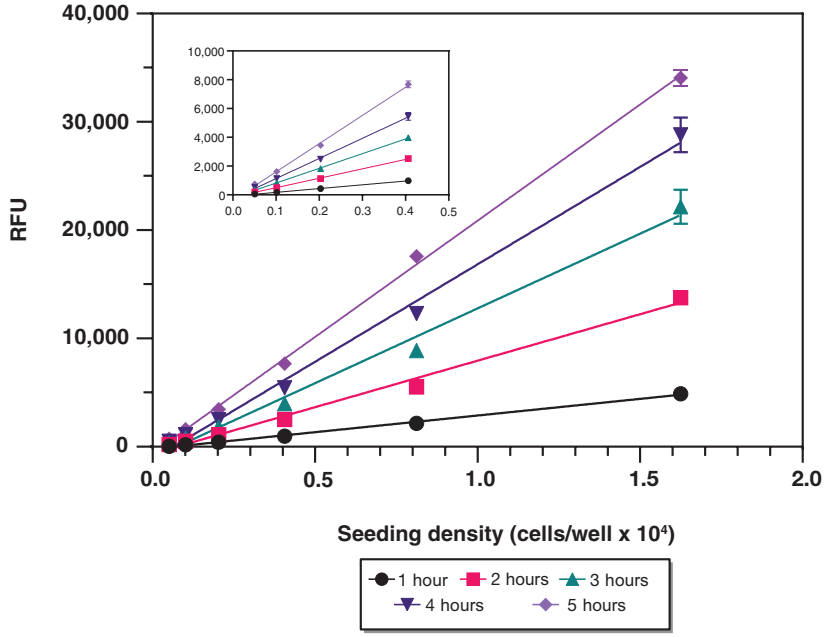

Figure 2. Typical cell titration experiment using A549 cells. Cells were plated in a titration series in 100 µl of medium and incubated overnight for attachment. Resazurin Cell Viability Reagent was added at a volume of 10 µl and fluorescence intensity was measured at different time intervals. A magnified image up to 0.4 x 10^4 cells/well is included for clarity.

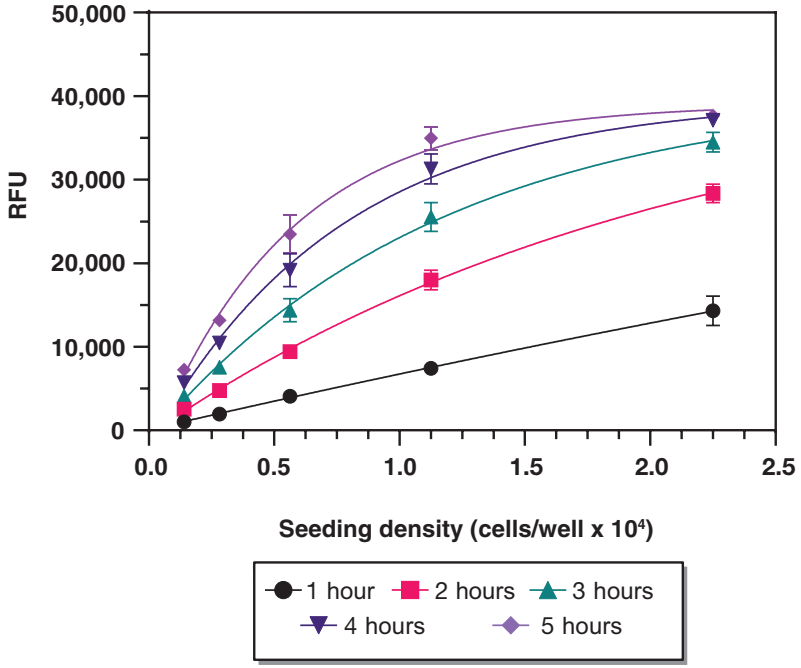

Figure 3. Typical cell titration experiment using HepG2 cells. Cells were plated in a titration series in 100 µl of medium and incubated overnight for attachment. Resazurin Cell Viability Reagent was added at a volume of 10 µl and fluorescence intensity was measured at different time intervals.

RESOURCES

Troubleshooting

Problem	Possible Causes
Low or absent signal/ no difference between background and experimental wells	A. Cells were seeded at too low of a density B. Not long enough of an incubation period
Signal exceeds instrument range	A. Cells were seeded at too high of a density B. Too long of an incubation period
Erratic values, dispersion of duplicates, or poor agreement of replicates	A. Cells were inconsistently seeded B. Reagents were not consistently added

References

1. Gonzalez, R.J. and Tarloff, J.B. Evaluation of hepatic subcellular fractions for Alamar blue and MTT reductase activity. *Toxicol. In Vitro* **15(3)**, 257-259 (2001).
2. O'Brien, J., Wilson, I., Orton, T., *et al.* Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **267(17)**, 5421-5426 (2000).
3. Pagé, B., Pagé, M., and Noël, C. A new fluorometric assay for cytotoxicity measurements *in vitro*. *Int. J. Oncol.* **3(3)**, 473-476 (1993).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.