Quick Cell Proliferation Testing Solution

Technical Manual No. 0221 **Version 10112010**

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I. DESCRIPTION

Quick Cell Proliferation Testing Solution provides the easiest and highest sensitive method for cell viability and toxicity assays. Quick Cell Proliferation Testing Solution uses nonradioactive reagent to determine the number of viable cells, for this reagent can be reduced by dehydroenases in living cells to give rise to a color change of the reagent, which can be read at 450nm of spectrophotometer of microplate reader. The absorption is directly proportional to the cell number which allows an accurate quantification of cell proliferation, viability, and cytotoxicity assays. Quick Cell Counting Solution enables cell counting easier and more sensitive than MTT, XTT, MTS based assays.

II. KIT COMPONENTS

Quick Cell Proliferation Testing Solution contains enough reagents for 1,000 assays, if a standard 96 well plate is used.

Components	1,000 Assays
Quick Cell Proliferation Testing Solution	50 ml
Protocol	1

III. APPLICATIONS

- Cell proliferation assay
- Cytotoxicity Assay

IV. KEY FEATURES

- Easy-to-use
- Safety, no radioactive or toxic reagent required

- More stable, sensitive and accurate than other cell counting methods

V. STORAGE

- Quick Cell Proliferation Testing Solution is stable for 2 years at -20°C and up to 2 months at 4°C.
- Protect from light.
- Avoid repeated freeze-thaw cycles.

VI. PROTOCOL

Cell Proliferation Assay Procedures:

1. The day before, culture cells in a 96-well plate in a final volume of 100 µl/well, according to the experimental factors tested.
2. Add 50 µl of Quick Cell Counting Solution to each well of the plate and mixture gently. Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.
3. Incubate the plate for 2-4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader with ~650 nm of reference filter.

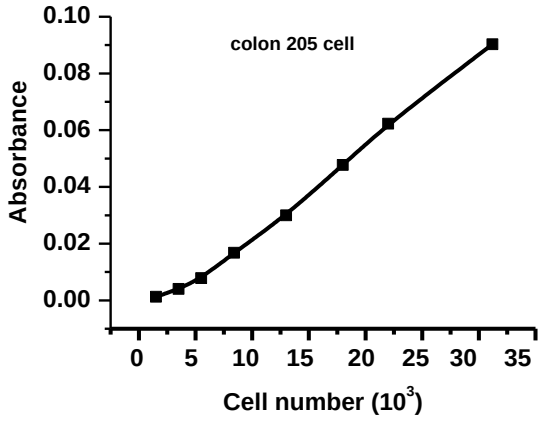
Background Control

1. There may be slight spontaneous absorbance at 460 nm occurs in culture medium. This background absorbance depends on the culture medium, pH, incubation time and length of exposure to light. To correct for this, prepare one or more control wells without cells, and subtract the average absorbance of the control wells from that of the other wells.
2. During a 4-hour experiment, the absorbance of the Quick Cell Proliferation Testing Solution does not increase at room temperature.

VII. EXAMPLES USING THE KIT

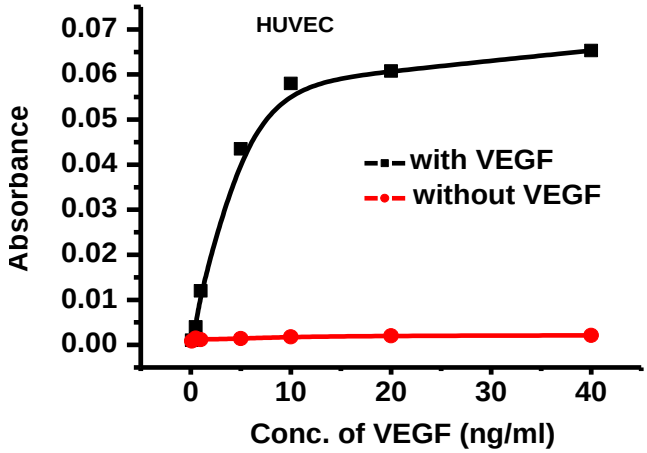
1. Cell Proliferation Assay

A. Spontaneous proliferation of colon205



(1) Varying numbers of colon205 cell suspension in a 96-well plate (100 µl/well) and incubate the plate in a humidified incubator at 37°C, 5% CO_2 for 48 hours.
(2) Add 50 µl of the Quick Cell Counting Solution to each well of the plate.
(3) Incubate the plate for additional 3-4 hours.
(4) Measure the absorbance at 450 nm using a microplate reader.

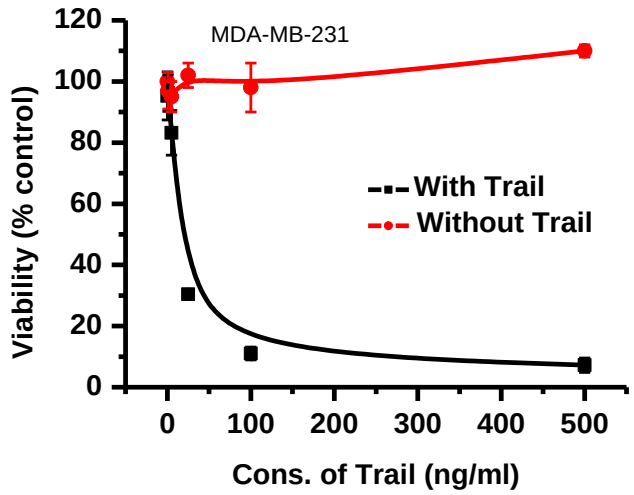
B. HUVEC Proliferation Stimulated by VEGF



(1) HUVEC cells in a collagen coated 96 well plate at a density of 2,000 per well and incubate for 12 hours.
(2) Add VEGF with a variety of concentrations.
(3) After two days, refeed the cells.

(4) On day 4, determine cell number with Quick Cell Counting Solution described above.

2. Cytotoxicity Assay



(1) Trail induced cytotoxity of MDA-MB-231. Seed 100 µl cells into a 96 well plate at a density of 10,000 cells per well and incubate.
(2) When the cells reach to 85% confluency, add Trail protein at a variety of concentrations.
(3) After 24 hours, determine cell number with Quick Cell Counting Solution described above.

VIII. TROUBLESHOOTING

1. How many cells should be in a well?

For adhesive cells, at least 1,000 cells are necessary per well (100 µl medium) when using a standard 96-well plate. If a 24-well or 6-well plate is used for this assay, the volume of Quick Cell Proliferation Testing Solution can be calculated proportional to the volume of media used per well.

3. Does Quick Cell Proliferation Testing Solution stain cells?

No, it does not stain cells. Thus Quick Cell Proliferation Testing Solution cannot be utilized for cell staining purpose.

3. Does phenol red affect the assay?

No. The absorption value of phenol red in a culture medium does not significantly interfere with the assay. Therefore, a phenol red containing medium is usable with Quick Cell Proliferation Testing Solution.

4. Is Quick Cell Counting Solution toxic to cells?

The toxicity of Quick Cell Proliferation Testing Solution is so low, so that, after the Cell Counting assay is completed, the same cells can be used for other assays such as the crystal violet assay, neutral red assay or DNA fluorometric assay.

5. I do not have a 450 nm filter. What other filters can I use?

You can use filters with the absorbance between 450 and 490 nm, even though 450 nm gives the best sensitivity.

IX. ORDER INFORMATION

Quick Cell Proliferation Testing Solution, Cat. No.: L00235

For Research Use Only.

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