KRISHGEN BioSystems

KineticBlue™ Cell Proliferation / Cell Viability-Cytotoxicity Assay

Catalog Number : CC1100

Description

KineticBlue™ Assay Kit offers a simple, rapid, reliable, sensitive, safe and cost-effective quantitation of proliferation of various human and animal cell lines, bacteria and fungi. KineticBlue™ detects cell viability by utilizing a blue and nonfluorescent dye resazurin, which is converted to a pink and fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. Continued cell growth maintains a reduced environment while inhibition of growth maintains an oxidized environment. Reduction related to growth causes the REDOX indicator to change from the oxidized (nonfluorescent, blue) form to the reduced (fluorescent, red) form. The fluorescent signal is monitored using 530-560 nm excitation wavelength and 590 nm emission wavelength. The absorbance is monitored at 570 nm and 600 nm. For optimal result, subtract background OD at 600 nm from OD at 570 nm. The fluorescent and colorimetric signal generated from the assay is proportional to the number of living cells in the sample. The KineticBlue™ assay is designed to quantitatively measure the proliferation of various human and animal cell lines, bacteria and fungi.

KineticBlue™ assay is as sensitive as thymidine assay for detecting cell proliferation. Depending on the cell types, KineticBlue can detect as few as 40 cells with reproducible and sensitive signal. Considering resorufin (pink and fluorescent) can be further reduced to hydroresorufin (uncolored and nonfluorescent), the assay signal decreases even with increased number of cells after all resazurin is converted into resorufin. Therefore, it is important to conduct a cell number titration assay for each particular cell line of your interest to identify the optimal number of cells for your assay to avoid this potential pitfall.

KineticBlue™ assay may also be used to measure relative cytotoxicity of agents.

Kit Component

30025-1
100mL KineticBlue™ solution (sterile)

KineticBlue™ Assay
Experimental Protocol

A) Standard Curve

1. Plate cells in 100µL medium into 96-well tissue culture plates by conducting cell number titration in the range of 40 to 10,000 for adherent cells and 2,000 to 500,000 for suspension cells. For background control, use 100µL medium without cells.
2. Add 10µL KineticBlue™ solution into medium and incubate cells at 37°C overnight.
3. Measure absorbance at 570 nm and 600 nm or fluorescence with excitation wavelength at 530 nm and emission wavelength at 590 nm using a micro-titer plate reader.
4. Obtain OD_{570}-OD_{600} for each sample if colorimetric detection method is chosen, or fluorescence signal from each sample deducted by background fluorescence from the background control, and plot a standard curve to identify the optimal cell concentration for your assay.

B) Assay Procedure

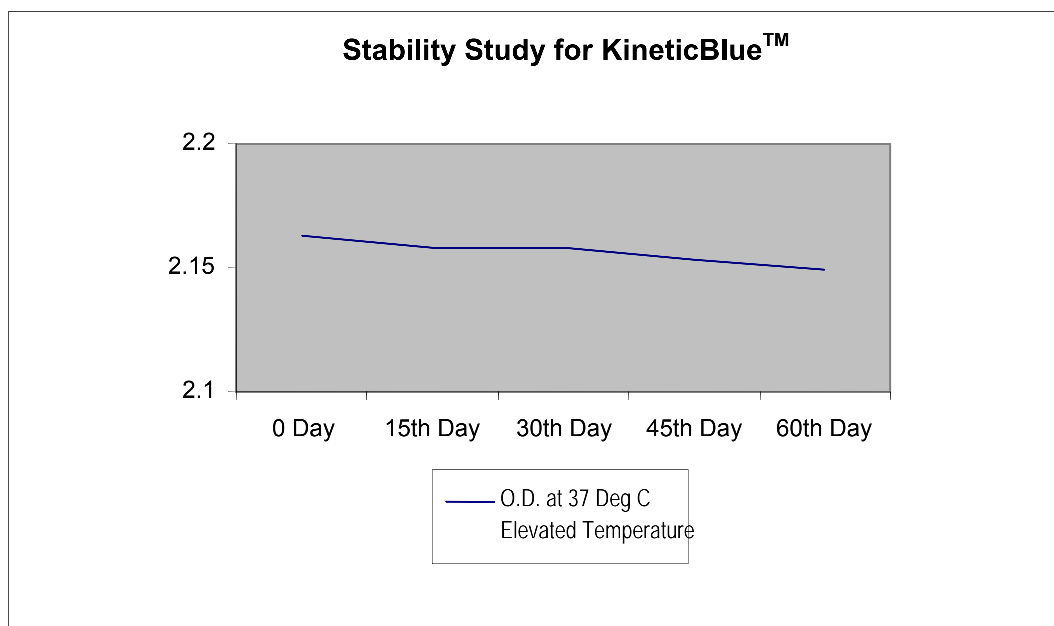
1. Plate cells into 96-well tissue culture plates using optimal cell concentration as derived in (A) above.

2. Carry out your experiment by adding agents of your interest into appropriate well and incubate with cells for a certain period of time.
3. Add 10µL KineticBlue™ solution into medium and incubate cells at 37°C overnight.
4. Measure absorbance at 570 nm and 600 nm or fluorescence with excitation wavelength at 530 nm and emission wavelength at 590 nm using a micro-titer plate reader.
5. Obtain OD_{570}-OD_{600} for each sample if colorimetric detection method is chosen, or fluorescence signal from each sample deducted by background fluorescence from the background control.

Storage and Handling

Upon receipt, the kit should be stored at 4°C and protected from light as the active component is light sensitive. Stored properly, the kit components should remain stable for 12 months.

Stability Study Of KineticBlue™					
Particulars	**Duration**				
Storage Condition at 37°C elevated temperature	**0 Day**	**15th Day**	**30th Day**	**45th Day**	**60th Day**
O.D. at 660 nm	**2.163**	**2.158**	**2.158**	**2.153**	**2.149**



Calculations For Determination Of Percentage Difference In Reduction Between Treated & Control Cells In Cytotoxicity / Proliferation Assays

Procedure 1 :-

- A correct factor **(RO)** for the absorbance of oxidized ***KineticBlue*** must be calculated.
- Measure the absorbance (AM) of growth medium alone. (Without addition of ***KineticBlue***)
- Measure the absorbance of oxidized (blue) ***KineticBlue*** in growth medium at the low and high wavelengths.
- Subtract (AM) from each of the measured ***KineticBlue*** absorbance to produce, respectively, AOLW and AOHW. These are absorbance of oxidized (blue) ***KineticBlue*** at the low and high wavelengths respectively.
- Calculate the correction factor RO of oxidized ***KineticBlue*** :

$$(RO) = (AOLW) / (AOHW)$$

- Measure the absorbance values (ALW and AHW) of a test sample at each wavelength.
- Calculate the percentage of reduced ***KineticBlue*** (ARLW) in a sample as :

$$ARLW = 100 \times [ALW-(AHW \times RO)]$$

- Calculate the percentage difference in reduction (PDR) between treated & control cells :

$$PDR = 100 \times (\textit{test}\ ARLW / \textit{positive growth control}\ ARLW)$$

Procedure 2 :-

To calculate the percent difference in reduction between treated and control cells in cytotoxicity/proliferation assay the following equation can be used :-

$$\%\ viability = [(£'_{600} \times A_{570}) – (£'_{570} \times A_{600})] \times 100/ [(£'_{600} \times C_{570}) – (£'_{570} \times C_{600})]$$

$$\%\ inhibition = 100 - \%\ viability$$

$£'_{570}$ = Molar extinction coefficient of *oxidized* ***KineticBlue*** at 570nm
$£'_{600}$ = Molar extinction coefficient of *oxidized* ***KineticBlue*** at 600nm
A_{570} = Absorbance of test wells at 570nm
A_{600} = Absorbance of test wells at 600nm
C_{570} = Absorbance of control well at 570nm
C_{600} = Absorbance of control well at 600nm

Molar Extinction Coefficients (ε) for KineticBlue -

Wavelength (λ)	ε Reduced	ε Oxidized
540 nm	17486	16281
570 nm	11054	24899
600 nm	2910	24396
630 nm	1785	36576

Note: *Wavelengths 540nm and 630nm can be used instead of 570nm and 600nm respectively.*

Example Calculation –

$\lambda_1 = 570nm$ $\lambda_2 = 600nm$ $£'_{570} = 24899$ $£'_{600} = 24396$
$A_{570} = 0.70$ $A_{600} = 0.41$ $C_{570} = 0.83$ $C_{600} = 0.24$

Using the Equation

% viability = [(£'$_{600}$ x A$_{570}$) – (£'$_{570}$ x A$_{600}$)] x 100/ [(£'$_{600}$ x C$_{570}$) – (£'$_{570}$ x C$_{600}$)]

$$= \frac{[(24396 \times 0.70) - (24899 \times 0.41)] \times 100}{[(24396 \times 0.83) - (24899 \times 0.24)]}$$

= 0.48 x 100
= 48%

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This would indicate that the amount of reduction in the test well is only 48% of that in the control well.

% inhibition = 100 - % viability
= **100 – 48 %**
= **52%**

Therefore, growth in the test well is inhibited by 52% when compared to that in the control.

Calculations To Determine The Percent Reduction Of KineticBlue

The following equation can be used –

$$\% \text{ reduced} = \frac{(£'_{600} \times A_{570}) - (£'_{570} \times A_{600})}{(£^{\#}_{570} \times C_{600}) - (£^{\#}_{600} \times C_{570})} \times 100$$

$£'_{570}$ = Molar extinction coefficient of oxidized Kinetic Blue at 570nm
$£'_{600}$ = Molar extinction coefficient of oxidized Kinetic Blue at 600nm
$£^{\#}_{570}$ = Molar extinction coefficient of reduced Kinetic Blue at 570nm
$£^{\#}_{600}$ = Molar extinction coefficient of reduced Kinetic Blue at 600nm
A_{570} = Absorbance of test wells at 570nm
A_{600} = Absorbance of test wells at 600nm
C_{570} = Absorbance of negative control well at 570nm
C_{600} = Absorbance of negative control well at 600nm

Example Calculation –

λ_1 = 570nm
λ_2 = 600nm
$£'_{570}$ = 24899
$£'_{600}$ = 24396
$£^{\#}_{570}$ = 11054
$£^{\#}_{600}$ = 2910.1
A_{570} = 0.69
A_{600} = 0.51
C_{570} = 0.54
C_{600} = 0.73

Using the above equation, the percent reduction of Kinetic Blue is -

$$\% \text{ reduced} = \frac{(24396 \times 0.69) - (24899 \times 0.51)}{(11054 \times 0.73) - (2910.1 \times 0.54)} \times 100$$

$$= 0.63 \times 100$$
$$= 63\ \%$$

References

Ahmed SA, Gogal RM Jr, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [3H] thymidine incorporation assay. J Immunol Methods. 1994 Apr 15;170(2):211-24.

Shahan TA, Siegel PD, Sorenson WG, Kuschner WG, Lewis DM. A sensitive new bioassay for tumor necrosis factor. J Immunol Methods. 1994 Oct 14;175(2):181-7.

Nociari MM, Shalev A, Benias P, Russo C. A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. J Immunol Methods. 1998 Apr 15;213(2):157-67.

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