Product Information

ApexBlue™ Quick Cell Viability Fluorometric Assay Kit

I. Kit Contents:

Component	K2023-500 500 assays	K2023-2500 2500 assays	Part Number
ApexBlue™ Reagent	5 ml	25 ml	K2023-C-1

II. Introduction:

Resazurin is a blue redox dye with weak fluorescent. When reduction by viable metabolically active cells, the dye becomes pink colored and highly red fluorescent.

ApexBlue™ Quick Cell Viability Fluorometric Assay Kit provides the easiest and most sensitive way for quantification of cell proliferation and viability. The metabolically active cells reduce blue resazurin into pink colored resorufin, which has high fluorescent (Ex = 530-570 nm; Em = 590-620 nm) and can be easily measured. The assay is very simple and only simply add the reagent to cell culture, incubate and read the fluorescence intensity.

III. General considerations:

1. Phenol red or serum does not interfere with the ApexBlue™ assay.
2. The assay can be performed in any type of culture plates, adjust the ApexBlue™ reagent amount to 10 % of culture medium. Duplicate or triplicate assays are recommended.
3. Drugs or compounds should be dissolved in PBS or culture medium, or perform proper solvent control if compound is dissolved in other solvents.

IV. Reagent Storage and handling:

Store the reagent at -20℃, stable for 1 year, protect from light. For research use only!

V. Cell Proliferation Assay Procedures:

1. Plate cells (1 - 5 x 10^5/well) in a 96-well microtiter plate in a final volume of 100 µl/well culture medium. For toxicity assays, use more cells to start with.

Notes:

1) The optimal cell number used for the assay may vary among cell types. For best results, it is recommended to add various numbers of cells in your initial assay to determine the optimal cell number to be used.

2) We recommend performing a reagent fluorescence background control by using the same amount of culture medium and ApexBlue™ Reagent without any cells.

2. Treat cells with your stimuli or drug for desired period of time (e.g. 12 - 96 hours).
3. Accurately add 10 µl (10 % medium volume) ApexBlue™ Reagent into each well, mix well gently. Be careful not to introduce bubbles to the wells.
4. Incubate the plate for 1 - 5 hours in standard culture conditions.

Note: Incubation time is dependent on cell type and cell number used. You may read the plate multiple times as desired and choose the best reading results.

5. Measure fluorescence intensity on a fluorescence plate reader, or fluorometer at Ex = 530 - 570 nm, Em = 590 - 620 nm.

Frequently Asked Questions

1. I see that it says the plate can be repeatedly read. Does this mean over the course of many days? I would like to compare the proliferation rates of 4 cell lines (wild type compared to stable cell lines expressing our gene of interest). I was wondering how best to do this using your kit. Can I plate out the cells and then continue to read the same wells every other day for two weeks? Or do I need to have wells for each time point?

No, the plate and the colour development cannot be read over a period of days. It is within the 4 hr incubation range, (or maybe slightly more than the 4 hrs if the desired OD is not achieved) after which the stop solution needs to be added. The plate can then be read within 48 hrs of stopping the colour development. In short, you need to have different wells for each time point. We would do the staining for all time points at the same time.

2. Hello, We are currently using a Quick cell proliferation assay kit II in our lab and I would like to know the minimum sensitivity (ie OD) the kit can detect. Below which OD value can we consider the reading as non-relevant (even if we substract an initial background)?

If we understand correctly, you are looking for an absolute cut-off value for the OD. This is a comparative assay, in which you can let the colour develop for an extended period of time to look at minor differences between various samples. The client will have to do their own statistical significance analysis on the differences they get form the use of this kit. They have to deem the control sample's OD at 100% cellular proliferation or 0% cell toxicity and go from there in their comparative analysis.

3. My customer is using this kit to check the cell proliferation however they say the colour development does not stop even after 5 hours of incubation. Customer is asking what sort of data they could get from it and for how long the intensity will go higher and higher.

Yes, the color might develop with longer incubation of the samples. However, the machine can detect the absorbance only within a certain limit. Keeping that in mind, we recommend repeated reads and termination of the reaction by adding the stop buffer whenever the desired reading is achieved. The 0.5-4 hrs is just the expected time range within which this is expected to happen.

4. In the protocol Step 4, it says "incubate in standard culture conditions". Is this 4℃ or RT?

In step 4, standard conditions refers to the conditions in which cells grow, e.g. in an incubator at 37℃.

5. Is there any alternative buffer to dissolve the WST reagent (in case something happens to the ECS solution)?

Please dissolve WST reagent in ECS solution only. If something happens to ECS, consider buying more of just ECS.

6. Is the kit compatible with all media types? (DMEM, Serum Free, etc…)

Yes, it is compatible with all media types, as long as your control untreated cells also grow in the same media.

7. Can this kit be used for both adherent and floating cells?

Yes, it is compatible with adherent and suspension cells.

8.What does the stopping solution contain? Is it basic or acidic solution or the enzymatic stopping is based on something else than pH?

The stop solution is detergent based.

9. Is this reagent WST also based on mitochondrial dehydrogenase activity? Could you please confirm?

Yes, this kit is based on mitochondrial dehydrogenase activity.

10. I am very surprised that this reagent (ECS) is not very toxic. MTT is very toxic.

ECS reagent does not contain the toxic MTT.

11. Can this kit be used with samples like bacteria, plants, drosophila, yeast etc?

We have optimized the kit with mammalian samples. However, theoretically these kits should work with samples from multiple species/sources. Since the optimal conditions depend on the sample type, the protocol has to be be adapted to fit the samples for efficient results. Please refer to this kits citations to see what kind of samples have been used with this kit other than mammalian samples.

12. Can we use frozen samples with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

13. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. You have to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

14. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

15. Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions etc)?

Our assay buffers are optimized for the reactions they are designed for. They not only contain some detergents for efficient lysis of your cells/tissue, but also contain some proprietary components required for the further reactions. Therefore, we highly recommend using the buffers provided in the kit for the best results.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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