Product Information

WST Cell Proliferation Colorimetric Assay Kit plus

I. Kit Contents:

Components	K2022-500 500 assays	K2022-2500 2500 assays	Part Number
WST Reagent (lyophilized)	1 vial	1 vial	K2022-C-1
Electrocoupling Solution	5 ml	25 ml	K2022-C-2
Stop Solution	5 ml	25 ml	K2022-C-3

II. Introduction:

Cell proliferation can be detected by a variety of methods. Cell proliferation induces an increase in the activity of the mitochondrial dehydrogenases, which cleaves the tetrazolium salt to formazan.

The WST Cell Proliferation Colorimetric Assay Kit plus provides the easiest and most sensitive way for quantification of cell proliferation and viability. Cell proliferation causes the increase in the amount of formazan dye formed that is directly proportional to the number of living cells and can be quantified by measuring the absorbance of the dye solution at 440 nm using microtiter plate reader. The kit can be used for the analysis of cell proliferation in response to pharmaceutical compounds, cytotoxic compounds like anticancer drugs and many other toxic agents. The assay can also be used for the detection of cell proliferation in response to cytokines, mitogens, growth factors and nutrients, etc. The assay is very easy and simple, just add-and-read, requiring no solubilization, no washing and no harvesting steps, and is stable, faster and more sensitive than MTS, MTT or XTT-based assays. The entire assay can be performed in a microtiter plate and correlates well with the [^{3}H]-thymidine incorporation assay.

III. Reagent Preparation and Storage:

Dissolve the lyophilized WST reagent into 5 ml the Electro Coupling Solution (ECS) for K2022-500 & 25 ml of ECS for K2022-2500. Aliquot the solution (1 ml is sufficient for one 96well plate assays) and store at –20℃. The WST solution is stable for 1 year at –20℃ and up to 6 months at 4℃. Protect from light.Avoid repeated freeze-thaw. Repeated freeze thaw may increase background.

IV. Cell Proliferation Assay Procedures:

1. Culture cells (0.1 - 5x10^4/well) in a 96-well microtiter plate in a final volume of 100 µl/well culture medium in the absence or presence of various amounts of the factors tested. For toxicity assays, use more cells to start with (e.g., 5x10^4 - 5x10^5 cells/well).

Note: 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 and the developing time to be used.

2. Incubate cells for 24 - 96 hours.

3. Add 10 µl per well WST reagent to each well. Be careful not to introduce bubbles to the wells.

4. Incubate the cells for 0.5 - 4 hours in standard culture conditions.

5. Shake thoroughly for 1 min on a shake. Measure the absorbance of the treated and untreated samples using a microtiter plate reader at 420-480 nm according to the filters available for the plate reader. The reference wavelength should be ~650nm

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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