Ver.260801

SRB Cytotoxicity Assay Kit

ORGM1000

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Product Description

The Sulforhodamine B (SRB) colourimetric assay is primarily used to detect cell proliferation. SRB is a pink anionic dye that is readily soluble in water and can specifically bind to basic amino acids of intracellular proteins under acidic conditions. It produces an absorption peak at 515nm wavelength, and the absorbance value exhibits a linear positive correlation with cell quantity, thus enabling quantitative detection of cell numbers.

Unlike the MIT assay, SRB does not change colour easily after staining. After cell fixation and staining, the 96-well plates can be stored for extended periods, making this method less affected by measurement timing. SRB dissolved in Tris solution also remains stable for prolonged durations Therefore, 96-well cell culture plates fixed at different time points can be measured simultaneously without significant impact on the absorbance results. When plotting absorbance values against SRB concentration, linearity is maintained below 1.5-2.0 OD units, when exceeding this linear range, samples should be diluted and re-measured. Although he SRB method involves more cumbersome operational steps compared to other detection methods, its flexibility in timing without time constraints makes it particularly suitable for high-throughput screening applications.

Kit components

Component	100T	Storage
Fixation Solution	10mL	2-8°C, avoid light
Wash Buffer 1	60mL	2-8°C
Wash Buffer 2	100mL	2-8°C
Staining Solution	10mL	2-8°C, avoid light
Dissolution Solution	20mL	2-8°C

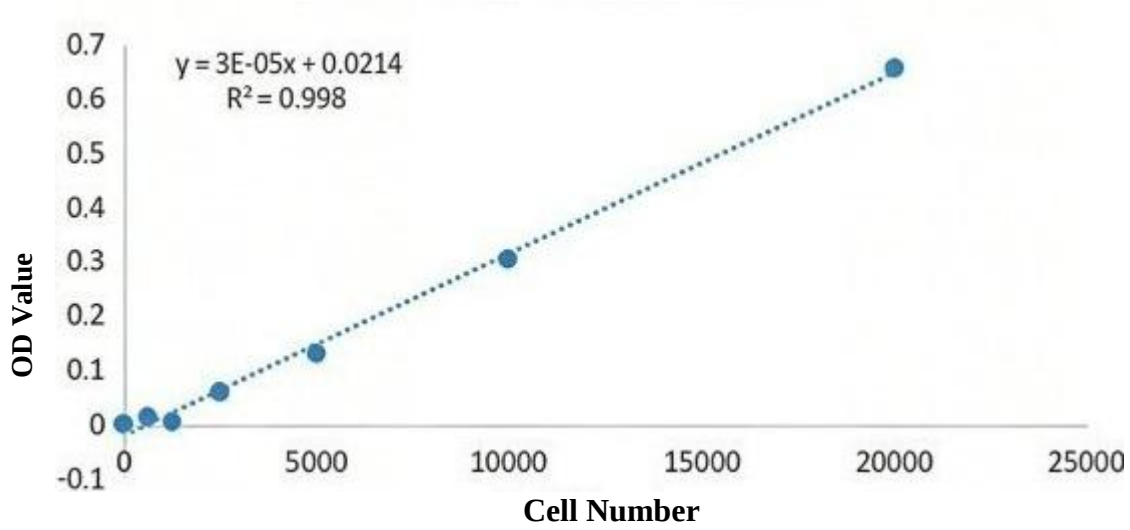
Protocol

1. Collect cells in the logarithmic growth phase, adjust the cell suspension concentration, and distribute into 96-well plates at 100µl per well.
2. Incubate at 37°C in a 5% CO: incubator to allow cell attachment, and culture for 6-24 hours.
3. Remove the plates from the incubator, discard the culture medium, and wash with PBS 1-2 times.
4. Add 100µL of pre-cooled fixation solution, stand at room temperature for 5 minutes, then stand at 4°C for 1 hour.
5. Discard the supernatant, wash three times with Wash Solution 1, and air dry at room temperature.
6. Add 100µL of staining solution, and incubate in the dark for 20 minutes (the plate can be wrapped with aluminum foil and placed on a horizontal shaker or orbital shaker for gentle shaking), then discard the staining solution.
7. Wash five times with Wash Solution 2 (complete quickly to prevent dye leakage from cells). remove residual staining solution as thoroughly as possible, and air dry at room temperature.

8. Add 200μL of dissolution solution, and incubate in the dark for 30 minutes (the plate can be wrapped with aluminum foil and placed on a horizontal shaker or orbital shaker for gentle shaking).
9. Measure the absorbance at 515nm wavelength.

Figure:

SRB Assay Growth Curve



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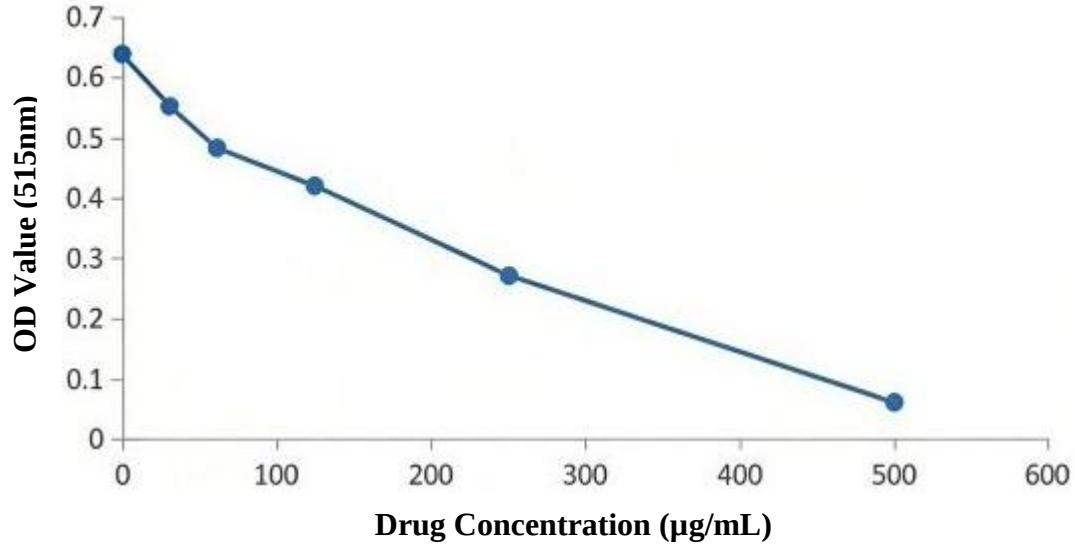

Notes

1. Prior to the formal experiment, please select several samples for pilot experiments to optimize experimental conditions, determine the optimal cell seeding density, and achieve the best experimental results.
2. Since a 96-well plate is used for detection, special attention must be paid to evaporation issues if cells are cultured for extended periods. This can be addressed by excluding the peripheral wells and replacing them with PBS, water, or culture medium; alternatively, placing the 96-well plate near the water source inside the incubator can help alleviate evaporation.
3. During seeding, ensure that the cell suspension is thoroughly mixed to prevent cell sedimentation, which may result in unequal cell numbers per well. It is recommended to mix the suspension after seeding every few wells.
4. For your safety and health, please wear a lab coat and disposable gloves when performing the experiment.
5. This product is for research use only. Do not use for medicinal, clinical diagnosis or therapeutic purposes, food, or cosmetics. Do not store in residential areas.