

# XTT Viability and Proliferation Assay Kit

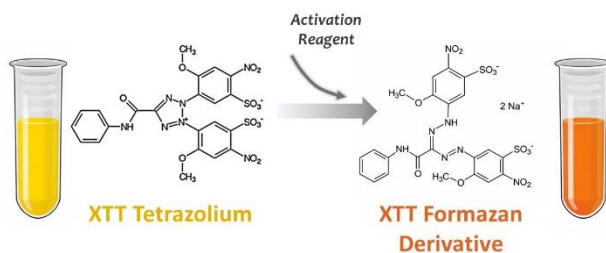
## Ordering info

TBK0501, 200 assays

TBK0502, 1.000 assays

## Description

**XTT Viability and Proliferation Assay Kit** is a colorimetric assay widely used to measure cellular viability, proliferation and cytotoxicity. It is based on the reduction of a yellow XTT tetrazolium salt to an orange soluble formazan derivative by the succinate dehydrogenase system of the mitochondrial respiratory chain.



The intensity of formazan dye is proportional to the number of living cells.

## Features

- **Improved efficiency**, with the addition of Activation Reagent as electron coupling in the reaction.
- **Higher accuracy**, based in the solubility of formazan derivative produced.
- **Highly sensitive**, low number of living cells could be measured.
- **Suitable for high throughput format**, homogeneous assay without washing steps.
- **Versatile**, valid for adherent and suspension cells.
- **Safe**, not require radioactive materials,

## Kit Components

Components	TBK0501	TBK0502
XTT Reagent	2 x 5 mL	10 x 5 mL
Activation Reagent	2 x 100 µL	10 x 100 µL

*Order Info Kit Components: XTT Reagent (TBR0257) | Activation Reagent (TBR0258).*

## Storage

Store the kit at  $-20^{\circ}\text{C}$  and protected from light. Stable for 1 year minimum.

## Quality Control

The kit is tested in a functional assay.

## Applications

- Determination of cell viability in presence of different agents.
- Cell proliferation.
- Cytotoxicity assays.

## Material required (not supplied)

- 96-well plates.

## PROTOCOL

1. Plate optimized number of cells in a 96-well plate containing a final volume of 100  $\mu\text{L}$ /well. Usually, a range density of  $5 \cdot 10^3$ -  $2 \cdot 10^5$  cells/ mL is optimal.
  - The culture media used may contain up to 10% serum and phenol red.
  - Include three wells with 100  $\mu\text{L}$  of growth medium alone as blank absorbance readings.
  - Use triplicates of the samples and control condition.
2. Carry out desired cell treatments.
3. Incubate the cell at 37 °C, 5% CO<sub>2</sub> for 24-48 hours.
4. Prepare fresh, immediately before its use, **XTT Working Solution**. For one 96 wells plate, mix 5 mL XTT Reagent and 100  $\mu\text{L}$  Activation Reagent. Protect from light.

*If sediment is observed in the XTT Working Solution, heat it to 37°C and swirl gently until a clear solution is obtained.*
5. Add **50  $\mu\text{L}$  XTT Working Solution** to each well. Mix gently.
6. Incubate for 2 hours (*adherent culture*) or for 4 hours (*suspension culture*).

*Incubation time must be optimized. It depends of cell type, number of cells, etc.*
7. Shake the plate gently to evenly distribute the dye in the wells.
8. Read the plates by absorbance at 450-500 nm (samples) and at 630-690 nm (background).
9. Calculated Absorbance normalized as follow:

$$\text{Absorbance normalized} = A_{450-500\text{nm}}(\text{sample}) - A_{450-500\text{nm}}(\text{blank}) - A_{630-690\text{nm}}(\text{background})$$

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$A_{\text{sample}} > A_{\text{control}}$	- Increase in cell proliferation.
	- Increase of viability.

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$A_{\text{sample}} < A_{\text{control}}$	- Decrease in cell proliferation
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