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Technical Service

IN VITRO TOXICOLOGY ASSAY KIT ACID PHOSPHATASE BASED

Stock No. TOX-3

For Research Use Only
Not for Use in Diagnostic Procedures

This kit provides a means for spectrophotometrically determining cell number as a function of membrane associated acid phosphatase.

**IT IS RECOMMENDED THAT THE ENTIRE
PROTOCOL BE REVIEWED BEFORE
STARTING THE ASSAY**

PRODUCT DESCRIPTION

Traditionally, the in vitro determination of toxic effects of unknown compounds has been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The acid phosphatase assay is a means of measuring the mass of cells via cell-membrane associated acid phosphatase.

The acid phosphatase method is simple, accurate and yields reproducible results. The key component is p-Nitrophenyl phosphate. Solutions of p-Nitrophenyl phosphate in medium or balanced salt solutions without phenol red are colorless. Cell-membrane associated acid phosphatase cleaves the substrate yielding a yellow colored compound (p-Nitrophenol) which is soluble in aqueous solutions. The resulting yellow solution is measured spectrophotometrically. An increase or decrease in cell numbers results in a concomitant change in the amount of substrate converted, indicating the degree of cytotoxicity caused by the test material.

KIT COMPONENTS

Catalog No.	Item	Quantity
N-4645	p-Nitrophenyl phosphate,	5 vials
	60 mg/vial	
P-5312	Acid Phosphatase Assay Buffer	25 ml

WARNING: Components in this kit should be carefully handled when using. The **p-Nitrophenyl phosphate** may be harmful if swallowed, inhaled or adsorbed through skin. **ACID PHOSPHATE ASSAY BUFFER** may be harmful if swallowed, inhaled or absorbed through skin.

PROCEDURE

The acid phosphatase method of monitoring in vitro cytotoxicity is a useful method that is well suited to multiwell plates. For best results, cells in the log phase of growth should be employed and final cell number should not exceed 10^6 cells/cm². Each test should include a blank containing complete medium without cells.

NOTE: Bacteria, mycoplasma and other microbial contaminants may also convert the p-Nitrophenyl phosphate substrate; thus cultures containing microorganisms should not be assayed using this method.

Acid Phosphatase activity can be assayed by either of the two methods described below. Method 1 provides greater sensitivity but cannot be used for suspended cell lines or cells which are loosely

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attached. Method 2 while less sensitive is less time consuming and can be used for all cell lines.

Method 1

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Prepare 30 ml of 1X Acid Phosphatase Assay Buffer by diluting 3 ml of 10X Assay Buffer with 27 ml of tissue culture grade water.
3. Prepare Acid Phosphatase Assay Mixture by adding the contents of one vial of p-Nitrophenyl phosphate to 30 ml of 1X Acid Phosphatase Assay Buffer. Aspirate off culture medium and add assay mixture in an amount equal to the original volume of culture medium.
4. Return cultures to incubator for 2-4 hours. An incubation period of 2 hours is generally adequate but may need to be lengthened for low cell densities or cells with low metabolic activity. Optimal incubation times should be determined and remain consistent when making comparisons.
5. Gentle stirring in a gyratory shaker will enhance mixing. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely disperse the reaction products.
6. Spectrophotometrically measure absorbance at a wavelength of 405 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract
6. Tests in multiwell plates can be read in an appropriate type of plate reader or the contents of individual wells transferred to appropriate size cuvetts for spectrophotometric measurement.

Method 2

1. Remove cultures from incubator into laminar flow hood or other sterile work area.
2. Reconstitute each vial of p-Nitrophenyl phosphate to be used with 3 ml of 10X Assay Buffer. Add reconstituted substrate in an amount equal to 10% of the culture volume.
3. Return cultures to incubator for 2-4 hours. An incubation period of 2 hours is generally adequate but may be lengthened for low cell densities or cells with low metabolic activity. Optimal incubation times should be determined

and remain consistent when making comparisons.

4. Gentle stirring in a gyratory shaker will enhance mixing. Occasionally, especially in dense cultures, pipetting up and down [trituration] may be required to completely disperse the reaction products.

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5. Spectrophotometrically measure absorbance at a wavelength of 405 nm. Measure the background absorbance of multiwell plates at 690 nm and subtract from 405 nm measurement.
6. Tests performed in multiwell plates can be read using an appropriate type of plate reader or the contents of individual wells may be transferred to appropriate size cuvetts for spectrophotometric measurement.

POSSIBLE SOURCES OF ERROR

1. Reconstituted p-Nitrophenyl phosphate solution and p-Nitrophenyl phosphate powder are stable when stored frozen. Storage at 4°C may result in decomposition and yield erroneous results.
2. Microbial contamination will contribute to degradation of p-Nitrophenyl phosphate yielding erroneous results.
3. Uneven evaporation of culture fluid in wells of multiwell plates may cause erroneous results.
4. Media and salt solutions with phenol red can be used but will contribute to higher background absorbance and can decrease sensitivity.

REFERENCE

Martin, A. and Clynes, M. [1991] Acid phosphatase: endpoint for in vitro toxicology tests. *In Vitro Cell Dev. Biol.* 27A:183-184.

Acid Phosphatase Based
TOX-3
2H125

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