pNPP ELISA Substrate System (ELPN-500)

For Rapid Accurate Alkaline Phosphatase and ELISA Assays

DESCRIPTION

Para-nitrophenyl phosphate (pNPP) is a chromogenic substrate for most phosphatases such as alkaline phosphatases and acid phosphatases. The reaction yields *para*-nitrophenol, an intense yellow product that can be conveniently measured at 405 nm on a spectrophotometer. The pNPP ELISA Substrate System has been optimized for enzyme-linked immunosorbent assays (ELISA) which use alkaline phosphatase conjugated antibody.

APPLICATIONS

ELISA Assays with alkaline phosphatase conjugated antibody.
Characterization of Alkaline Phosphatase: determination of phosphatase activity and phosphatase mechanism of action.
Drug Discovery: high-throughput screen for inhibitors of alkaline phosphatase.

KIT CONTENTS

Catalog #	No. of Tests	Reagent	Buffer
ELPN-500	500	solid	100 mL

Storage conditions. Store the Reagent and Buffer at -20°C and at 4°C, respectively. Shelf life: 12 months.

This protocol can be downloaded online at www.bioassaysys.com.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES (96-WELL PLATE)

1. Reconstitute the Substrate system. Fresh reconstitution is recommended although the reconstituted Substrate may be stable for up to 4 weeks when stored at -20°C. Simply add 100 mL Buffer to the amber Reagent bottle. Mix thoroughly by inversion multiple times. After this is done, mark the bottle label as Reconstituted Substrate. Equilibrate to room temperature before use, for all subsequent procedures are carried out at room temperature.

2. After the secondary antibody (alkaline phosphatase conjugate) has been incubated and thoroughly washed to remove unbound conjugate, add 200 µL of the reconstituted pNPP susbtrate per well. Incubate the plate in the dark for approximately 30 min.

3. Read the plate at 405 nm on a plate reader.

If the plate can not be read immediately, add 50 µL Stop Solution (3 N NaOH, not provided) to terminate further color reaction. Mix by quickly tapping the plate.

GENERAL CONSIDERATIONS

Background too high. A blocking step is preferred. This can be done by using a 1-5% serum prior to the application of the primary antibody. Adequate washes (including 0.1% TWEEN) are necessary to remove unbound antibody.

Color too faint. Weak color development occurs when primary/or secondary antibody concentrations are too low. A control experiment can be run to determine the activity of the alkaline phosphatase conjugate. Alternatively, allow the reaction to proceed for a longer period of time or at a higher temperature (e.g. 37°C).

LITERATURE

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