

## pNPP

## CHROMOGENIC SUBSTRATES

**ELISA AP Substrate**

Cat. No. 4400

Lot No.: XXXXXXDate of manufacture: XX-XXXXExpiry date: XX-XXXX (When stored at 2-8 °C in an unopened bottle)**Analysis:**

The substrate is analyzed for consistent activity, background, color and pH (only the procedure for activity and background is described in the COA).

**Background:** 100 µL substrate is pipetted into four wells of a standard microtiter plate, followed by addition of 100 µL 1M sodium hydroxide. The background absorbance is read at 405/620 nm.

**Activity:** The activity is determined in a 2-layer ELISA. First layer is a standard Human serum dilution. Second layer is Rabbit anti Human IgG-AP. 100 µL substrate is added to each well and incubated for 30 minutes. The reaction is stopped by addition of 100 µL 1 M sodium hydroxide. The absorbance is read at 405/620 nm.

The activity is compared to a standard p-NPP solution with known activity.

**Specifications:**

Activity: 90 - 110%

Background: Abs. &lt; 0.080

pH: 9.35 - 9.75

Color: Clear to slightly yellow

**Results:**

Activity test: \_\_\_\_\_

Background: \_\_\_\_\_

Color: \_\_\_\_\_

pH: \_\_\_\_\_

**Storage:**

Short-term storage: Ambient; protected from light.

Long-term storage: 2-8 °C; protected from light.

**Performed by:**Date: xx-xxx-2021Signature: xx**Approved by:**Date: xx-xxx-2021Signature: xx

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