F17-3333-3-C; Effective: 4/25/2017; Supersedes F17-3333-3-A

Stop Solution for AP Substrates (STOPP)

Stop and stabilize AP microwell substrate reactions.

Stop Solution for AP Substrates (STOPP) is a dry powder formulation used to stop alkaline phosphatase (AP) microwell substrate reactions without causing a color or absorbance change. It is suitable for all endpoint ELISAs using an alkaline phosphatase substrate reaction for color development.

AP substrates, such as pNPP, are oxidized by alkaline phosphatase to yield a soluble, colored reaction product. For example, a yellow reaction product is generated when pNPP 1-Component AP Microwell Substrate (SUBP, catalog #6279) reacts with alkaline phosphatase. In endpoint assays, the reaction can be stopped by adding equal volumes of Stop Solution for AP Substrates (STOPP). The use of STOPP does not cause a change in chromogen color, and the reaction is stable for one hour. When STOPP is used to stop pNPP substrate (SUBP, catalog #6279), the signal of the bright yellow reaction product can be read between 405-420 nm.

To avoid overdeveloping the AP substrate reaction, the absorbance should be periodically monitored on an ELISA plate reader using absorbance filter settings appropriate for the AP substrate (e.g., 405-420 nm for pNPP). When OD values reach approximately 2.0-2.5 units, the reaction should be stopped with STOPP. If the reaction product yields OD values above 2.5 units, it is recommended to dilute the conjugate or shorten the conjugate incubation period.

Reconstitute Stop Solution for AP Substrates by adding 100 mL of DiH_2O and mix well. Once reconstituted, STOPP is ready-to-use. Equal volumes of AP microwell substrate and stop solution should be used. Best results are obtained by equilibrating STOPP to room temperature (25°C) prior to use.

Build a better assay with ELISA Solutions from ImmunoChemistry Technologies.

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STOP SOLUTION FOR AP SUBSTRATES (STOPP)

Size	Catalog #
100 mL	#6284

INSTRUCTIONS:

1. Reconstitute STOPP by adding 100 mL DiH_2O and mix well.
2. Run ELISA according to the specific protocol through the conjugate incubation step.
3. Wash the wells three or four times with tris saline wash buffer to remove any residual AP-conjugate.
4. Add AP substrate to each well of the plate. For example, use 100 µL/well.
5. Incubate the substrate 10-60 minutes.
6. Monitor the color intensity on an ELISA plate reader. When OD values reach approximately 2.0-2.5 units, stop the reaction.
7. Stop the reaction by adding an equal volume of STOPP to the substrate in every well of the plate.
8. Read the plate within 1 hour at the absorbance appropriate for the AP substrate, e.g., read stopped pNPP at 405-420 nm.
9. Analyze data.

For more ELISA protocols and information, please visit www.immunochemistry.com.

SPECIFICATIONS:

- Dry powder (reconstitute with 100 mL DiH_2O)
- Clear, colorless liquid (when reconstituted)
- Read stopped pNPP reaction product between 405-420 nm.

STORAGE:

- Store at 25°C.
- Refrigerated temperatures will not harm the reagent.

SAFETY & USAGE:

- **Warning!** Harmful if swallowed, in contact with skin or if inhaled. Causes skin irritation. Causes serious eye irritation. May cause respiratory irritation.
- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only

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