

Buffer Pack

For Alkaline phosphatase
Coating, wash and substrate buffers
Catalog number: ACC 00113

List of contents

Lot number	Item	500 wells	1000 wells	5000 wells
_____	Carbonate coating buffer, 10X concentrate	30 ml	30 ml	2 x 30 ml
_____	PNP substrate buffer, 5X concentrate	25 ml	25 ml	125 ml
_____	PNP substrate tablets, 5 mg each	25 tablets	25 tablets	100 tablets
	<i>The above items should be stored at 4 °C</i>			
_____	PBST wash buffer, powder	110 g	110 g	5 x 110 g
	<i>The above items should be stored at room temperature.</i>			

Materials required but not provided

- Distilled water

Storing the reagents

Store all components at the recommended temperature (above) to assure their full shelf life.

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Preparing Buffers from concentrate or powder

Carbonate coating buffer Carbonate coating buffer is used to dilute the concentrated coating antibody. The volume of 1X carbonate coating buffer required depends on the number of test wells used, with 100 µl needed per test well. To estimate the volume needed, prepare 1 ml for each 8-well strip used, or 10 ml for each 96-well plate.

Dilute 1 volume of 10X carbonate coating buffer concentrate with 9 volumes of distilled water before use.

Example: To prepare 10 ml of working coating buffer mix 1 ml of 10X carbonate coating buffer concentrate with 9 ml of distilled water.

PNP substrate buffer PNP substrate buffer is used to dilute PNP substrate tablets. The volume of 1X PNP buffer required depends on the number of test wells used, with 100 µl needed per test well. An entire 96-testwell plate would require 10 ml.

Dilute 1 volume of 5X PNP buffer concentrate with 4 volumes of distilled water before use.

Example: To prepare 10 ml of working PNP substrate buffer, mix 2 ml of 5X PNP buffer concentrate with 8 ml of distilled water.

Each PNP tablet will make 5 ml of PNP solution, at a concentration of 1 mg/ml.

About 15 minutes before the end of the last incubation step, measure 5 ml of room-temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the buffer.

Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

PBST wash buffer Prepare 1X buffer by dissolving PBST buffer powder in water according to the table below:

Buffer powder	5 g
Distilled water	500 ml

or

Dilute 1 volume of 20X concentrated PBST stock solution with 19 volumes of distilled water before use.

20 X PBST concentrate To prepare a 20X concentrate of PBST:

Buffer powder	110 g
Distilled water	500 ml

Measure the distilled water into a container and add the buffer powder while stirring.

Store 20X PBST wash buffer in a closed container at room temperature. Shelf life is one year.

Optional: If you wish to check the pH: Prepare 20 ml of 1X PBST wash buffer by diluting 1 ml of stock solution with 19 ml of distilled water. Adjust the pH of the stock solution, if necessary, until the pH of the 1X buffer is within the range of 7.2 to 7.6.