

# User Guide: Buffer Pack

ACC 00111 • DAS and Compound Alkaline Phosphatase ELISAs

---

## Instructions

Prepare buffers fresh each day. Storing 1X buffers for extended periods comes with risks such as precipitation, growth, or contamination which can have an effect on test performance. For those willing to assume this risk Agdia has recommended storage conditions and preservatives where possible.

Agdia's powdered and liquid concentrate buffers are designed, when made following their protocol, to have a pH within the approved range. Checking the pH of the buffers can serve as a procedural check, but is not required, simply recommended.

## Handling Information

Components should be stored according to each product's label. All materials should be warmed to room temperature (18 - 30 °C) before use. For materials provided please see the product webpage.

## Safety

Agdia recommends reading all relevant SDS sheets before using assay components: <http://docs.agdia.com/DataSheets.aspx>.

## Carbonate Coating Buffer (CCB) (1X)

1. Carbonate Coating Buffer (CCB) is used to dilute capture antibodies.
2. CCB is provided as a 10X concentrate; mix well before use.
3. Dilute 1 volume of 10X CCB concentrate with 9 volumes of deionized or similar purity water before use.

*Example: To prepare 10 mL of 1X CCB, mix 1 mL of 10X CCB concentrate with 9 mL of water.*

4. **Optional:** Adjust the pH to the range of 9.5 to 9.7.
5. **Optional:** Store at 2 - 8 °C. Sodium azide is already present in buffer.

## General Extract Buffer (GEB) (1X)

1. General Extract Buffer (GEB) is used to grind and dilute samples.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare GEB powder in water according to the table below:

Powder	16.5 g
TWEEN® 20	10.0 g
Water <sup>1</sup>	500 mL

<sup>1</sup>Use deionized or similar purity water

4. Add approximately 100 mL of water to the powder and mix into a smooth slurry.
5. While mixing, slowly add the remaining volume of water and the TWEEN® 20 to the solution.
6. Stir for 15 to 30 minutes or until the powder is dissolved.
7. **Optional:** Adjust the pH to the range of 7.2 to 7.8.
8. **Optional:** Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.

## ECl Buffer (1X)

1. ECl is used to dilute enzyme conjugate antibodies.
2. ECl is provided as a 5X concentrate; mix well before use.
3. Dilute 1 volume of 5X ECl concentrate with 4 volumes of deionized or similar purity water before use.

*Example: To prepare 15 mL of 1X ECl, mix 3 mL of 5X ECl concentrate with 12 mL of water.*

4. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
5. **Optional:** Store at 2 - 8 °C. Sodium azide is already present in buffer.



## PBST Buffer (1X) (From Powder)

1. PBST Buffer is used to wash ELISA plates.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare PBST powder in water according to the table below:

Powder	5.0 g
Water <sup>1</sup>	500 mL

<sup>1</sup>Use deionized or similar purity water

4. Measure the water into a container and add the powder while stirring.
5. Stir for 15 to 30 minutes or until the powder is dissolved.
6. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
7. **Optional:** Store at 18 - 30 °C. Sodium azide is not recommended.

## PBST Buffer Liquid Concentrate (20X)

1. 20X PBST can be stored and diluted to 1X PBST buffer as needed.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare PBST powder in water according to the table below:

Powder	100.0 g
Water <sup>1</sup>	500 mL

<sup>1</sup>Use deionized or similar purity water

4. Measure the water into a container and add the powder while stirring.
5. Stir for 15 to 30 minutes or until the powder is dissolved.
6. Store at 18 - 30 °C. Sodium azide is not recommended. Shelf life is one year.

## Preparing 1X PBST from 20X PBST

1. Dilute 1 volume of 20X PBST concentrate with 19 volumes of deionized or similar purity water before use.  
*Example: To prepare 1000 mL of 1X PBST, mix 50 mL of 20X PBST concentrate with 950 mL of water.*
2. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
3. **Optional:** Store at 18 - 30 °C. Sodium azide is not recommended.

## PNP Substrate Buffer (1X)

1. PNP substrate buffer is used with PNP substrate tablets to make an active substrate for alkaline phosphatase ELISA systems.
2. PNP substrate buffer is provided as a two-component system, a 5X concentrate and 5 mg tablets; mix well before use.
3. Dilute 1 volume of 5X PNP substrate concentrate with 4 volumes of deionized or similar purity water before use.

*Example: To prepare 10 mL of 1X PNP substrate buffer, mix 2 mL of 5X PNP substrate concentrate with 8 mL of water.*

*Do not add PNP substrate tablets until the day of testing.*

4. **Optional:** Adjust the pH to the range of 9.7 to 9.9 with hydrochloric acid.
5. **Optional:** Store at 2 - 8 °C. Sodium azide is already present in buffer.

## On the day of testing prepare PNP Substrate Solution:

1. Add 1 PNP substrate tablet per 5 mL of 1X PNP substrate buffer into a dedicated container.  
*Example: To prepare 10 mL of 1X PNP substrate solution, add 2 PNP substrate tablets to 10mL of 1X PNP substrate buffer.*
2. Ensure tablets are dissolved before use.
3. Keep prepared 1X PNP solution in the dark prior to use.
4. Dispose of any remaining prepared PNP substrate at the end of the day.

## Warranty

Agdia reagents are warranted for performance issues that arise from manufacturer defect. See product packaging for relevant expiration dates. Agdia's return policy can be found at [www.agdia.com/customer-support/return-policy](http://www.agdia.com/customer-support/return-policy).

## Additional Information

If you would like more information on how to run ELISA, please see Agdia's FAQ section, <http://www.agdia.com/customer-support/frequent-questions-and-troubleshooting>. For further documentation, including this user guide, please see Agdia's specific product webpages. For answers to your technical questions, please contact us at [techsupport@agdia.com](mailto:techsupport@agdia.com).

TWEEN® is a registered trademark of Croda International PLC.

