

User Guide: Buffer Instructions

Powdered and Liquid Concentrate Buffers

Instructions

This user guide contains instructions for preparing all of Agdia's powdered and liquid concentrate buffers available for all pathogen ELISAs. Be sure to check the ELISA user guide for approved buffers.

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.

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 ¹	500 mL

¹Use 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.

General Extract Buffer 2 (GEB2) (1X)

1. General Extract Buffer 2 (GEB2) is used to grind and dilute samples.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare GEB2 powder in water according to the table below:

Powder	27.9 g
Water ¹	500 mL

¹Use 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.
6. Stir for 15 to 30 minutes or until the powder is dissolved.
7. **Optional:** Adjust the pH to the range of 8.0 to 8.2.
8. **Optional:** Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.



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General Extract Buffer 3 (GEB3) (1X)

1. General Extract Buffer 3 (GEB3) is used to grind and dilute samples.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare GEB3 powder in water according to the table below:

Powder	24.0 g
TWEEN® 20	10.0 g
Water ¹	500 mL

¹Use 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.

General Extract Buffer 4 (GEB4) (1X)

1. General Extract Buffer 4 (GEB4) is used to grind and dilute samples.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare GEB4 powder in water according to the table below:

Powder	16.5 g
TWEEN® 20	10.0 g
Water ¹	500 mL

¹Use 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 7.6.
8. **Optional:** Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.

PBST Buffer (Wash Buffer) (1X)

1. PBST is used to wash ELISA plates.
2. PBST is provided as a 20X concentrate; mix well before use.
3. Dilute the entire 50 mL of 20X PBST concentrate with 950 mL of deionized or similar purity water before use.
4. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
5. **Optional:** Store at 18 - 30 °C. Sodium azide is not recommended.

Indirect Sample Extraction Buffer (IEB) (1X)

1. Indirect Sample Extraction Buffer (IEB) is used to grind and dilute samples.
2. IEB is provided as a 10X concentrate; mix well before use.
3. Dilute 1 volume of 10X IEB concentrate with 9 volumes of deionized or similar purity water before use.

Example: To prepare 10 mL of 1X IEB, mix 1 mL of 10X IEB 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.

ECI Buffer (1X)

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

Example: To prepare 15 mL of 1X ECI, mix 3 mL of 5X ECI 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.



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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:

6. 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.
7. Ensure tablets are dissolved before use.
8. Keep prepared 1X PNP solution in the dark prior to use.
9. Dispose of any remaining prepared PNP substrate at the end of the day.

MEB Buffer (1X)

1. MEB is used to grind and dilute samples.
2. Prepare MEB with non-fat dried milk (NFDM) powder in 1X PBST according to the table below:

NFDM	2.0 g
TWEEN® 20	2.5 g
1X PBST	500 mL

3. Add approximately 100 mL of 1X PBST to the powder and mix into a smooth slurry.
4. While mixing, slowly add the remaining volume of 1X PBST and the TWEEN® 20 to the solution.
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 7.8.
7. **Optional:** Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.

ECM Buffer (1X)

1. ECM is used to dilute enzyme conjugate antibodies.
2. Prepare ECM with non-fat dried milk (NFDM) powder in 1X PBST according to the table below:

NFDM	0.4 g
1X PBST	100 mL

3. Measure the 1X PBST into a container and add the powder while stirring.
4. Stir for 15 to 30 minutes or until the powder is dissolved.
5. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
6. **Optional:** Store at 2 - 8 °C. Sodium azide is not recommended.

APLPV EC Buffer (1X)

1. APLPV EC is used to dilute APLPV enzyme conjugate antibodies.
2. Shake the bottle of powdered buffer before measuring to ensure components are thoroughly mixed.
3. Prepare APLPV EC powder in water according to the table below:

Powder	4.05 g
Water ¹	100 mL

¹Use 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. Do not heat the buffer to speed up the mixing process.
6. **Note:** This buffer contains healthy plant tissue that may not completely go into solution. Because of this, a large orifice pipette tip may be needed to dispense the buffer.
7. **Optional:** Adjust the pH to the range of 7.2 to 7.6.
8. **Optional:** Add sodium azide (Sigma S2002) at a rate of 0.2 g per liter (0.02 %) and store at 2 - 8 °C.



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PBS Buffer (1X)

1. PBS is used as the solvent in MPBS (BRA Blocking Buffer).
2. PBS is provided as a 20X concentrate; mix well before use.
3. Dilute 1 volume of 20X PBS concentrate with 19 volumes of deionized or similar purity water before use.

Example: To prepare 20 mL of 1X PBS, mix 1 mL of 20X PBS concentrate with 19 mL of water.

4. **Optional:** Adjust the pH to the range of 7.3 to 7.5.
5. **Optional:** Store at 18 - 30 °C. Sodium azide is not recommended.

MPBS Buffer (1X) (BRA Blocking Buffer)

1. MPBS is used to block Bacterial Reagent Set (BRA) ELISA plates.
2. Prepare MPBS with non-fat dried milk (NFDM) powder in 1X PBS according to the table below:

NFDM	1.0 g
1X PBS	20 mL

3. Measure the 1X PBS into a container and add the powder while stirring.
4. Stir for 15 to 30 minutes or until the powder is dissolved.
5. **Optional:** Store at 2 - 8 °C. Sodium azide is not recommended.

MPBST Buffer (1X) (BRA Antibody Diluent)

1. MPBST is used to dilute enzyme conjugate antibodies.
2. Prepare MPBST with non-fat dried milk (NFDM) powder in 1X PBST according to the table below:

NFDM	0.5 g
1X PBST	20 mL

3. Add the entire amount of powder to the 1X PBST.
4. Stir for 15 to 30 minutes or until the powder is dissolved.
5. **Optional:** Store at 2 - 8 °C. Sodium azide is not recommended.

MRS Component (1X)

1. MRS is used to dilute enzyme conjugate antibodies.
2. MRS is provided as a 5X concentrate; mix well before use.
3. Dilute 1 volume of 5X MRS concentrate with 4 volumes of 1X PBST before use.

Example: To prepare 15 mL of 1X MRS, mix 3 mL of 5X MRS concentrate with 12 mL of 1X PBST.

4. **Optional:** Adjust the pH to the range of 7.0 to 8.0.
5. **Optional:** Store at 2 - 8 °C. Preservative is already present in buffer.

MRS-2 Component (1X)

1. MRS-2 is used to dilute enzyme conjugate antibodies.
2. MRS-2 is provided as a 5X concentrate; mix well before use.
3. Dilute 1 volume of 5X MRS-2 concentrate with 4 volumes of 1X PBST before use.

Example: To prepare 15 mL of 1X MRS-2, mix 3 mL of 5X MRS-2 concentrate with 12 mL of 1X PBST.

4. **Optional:** Store at 2 - 8 °C. Preservative is already present in buffer.

PEB1 Extraction Buffer (1X)

1. PEB1 is used to grind and dilute samples.
2. PEB1 is provided as a 10X concentrate; mix well before use.
3. Dilute 1 volume of 10X PEB1 concentrate with 9 volumes of deionized or similar purity water before use.

Example: To prepare 10 mL of 1X PEB1, mix 1 mL of 10X PEB1 concentrate with 9 mL of water.

4. **Optional:** Adjust the pH to the range of 7.3 to 7.5.
5. **Optional:** Store at 2 - 8 °C. Preservative is already present in buffer.

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