F17-524-3-E; Effective 05/26/2017; Supersedes F17-524-3-D

Antibody Coating Buffer, 5X

Enhances adsorption of antibodies while preserving structure.

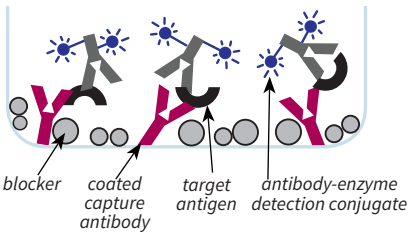
Antibody Coating Buffer, 5X is a protein-stabilizing solution that maximizes the adsorption of capture antibodies onto polystyrene plates. During the plate coating process, the salt and pH buffering environment provided by Antibody Coating Buffer stabilizes the three-dimensional antibody structure, preserving the antigen recognition regions of the antibody. This buffered environment also provides a highly consistent adsorption rate across all wells of the ELISA plate. In addition, this unique protein stabilization buffer may allow for the use of lower quantities of valuable capture antibody. Therefore, the use of Antibody Coating Buffer allows ELISA plates to be manufactured with high levels of precision and antigen capture utility.

Antibodies are typically coated onto ELISA plates at 1-10 µg/mL, using 50-200 µL of 1X coating solution per well. This range translates to approximately 1.1-4.4 mL of Antibody Coating Buffer, 5X per 96-well plate. To calculate the necessary amount of 1X coating solution, multiply the desired fill-volume per well by the number of wells. Prepare 10% extra as some of the solution will be lost during pipetting. For example, to coat 3 plates at 100 µL/well, calculate:

- 1X coating solution: 100 µL/well x 96 wells x 3 plates = 28.8 mL x 110% = 31.7 mL needed.
- Antibody Coating Buffer, 5X: 31.7 mL / 5 = 6.3 mL.
- DiH_2O required: 31.7 mL - 6.3 mL = 25.4 mL.
- Add 6.3 mL Antibody Coating Buffer, 5X to 25.4 mL diH_2O.
- Add the appropriate volume of capture antibody to the 1X coating solution to attain the target coating concentration.

As Antibody Coating Buffer is concentrated 5X, crystalline precipitates may form in the bottle, especially when refrigerated. If this happens, gently warm or mix the buffer until all crystals are dissolved. Plates may be coated at room temperature.

Antibody Sandwich ELISA



Build a better assay with ELISA Solutions from ImmunoChemistry Technologies.

BRIGHT MINDS, BRIGHT SOLUTIONS.

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

ANTIBODY COATING BUFFER, 5X

Size	Catalog #
100 mL	#644
500 mL	#645
1 L	#646
10 L	#658

INSTRUCTIONS:

1. Calculate the amount of 1X coating solution needed. ELISA plates are typically coated with 50-200 µL per well.
2. Mix Antibody Coating Buffer, 5X to dissolve any precipitates in the bottle; avoid bubbles. If necessary, gently warm the concentrated buffer until all crystals are in solution.
3. Dilute Antibody Coating Buffer 1:5 by adding 1 part coating buffer to 4 parts diH_2O.
4. Mix until crystals have dissolved.
5. Add the antibody to the 1X buffer at the target concentration. The optimal coating concentration typically ranges from 1-10 µg/mL.
6. Mix for 15 minutes.
7. Pipette the coating solution into each well of the microtiter plate, typically at 50-200 µL per well.
8. Incubate 8-24 hours at room temperature.
9. Aspirate the coating solution.
10. Wash each well twice with 1X ELISA Wash Buffer (catalog #652).
11. Block the uncoated regions of the microplate wells by pipetting 300-400 µL of blocking buffer (such as catalog #640) into each well.
12. Incubate 8-24 hours at room temperature.
13. Aspirate the blocking buffer.
14. Run the assay immediately, or dry the plate for long-term storage and seal in a foil bag (catalog #6288) with a desiccant pack (catalog #6289).

SPECIFICATIONS:

- Clear liquid
- 5X concentrate
- pH 7.8-8.2

STORAGE:

- 24 months at room temperature
- Product may also be stored at 2-8°C

SAFETY & USAGE:

- Contains ≤ 0.5% sodium azide
- SDS available at immunochemistry.com
- Not for human or drug use
- For research use only

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