F17-5136-3-D; Effective 05/26/2017; Supersedes F17-5136-3-C

Antigen Coating Buffer, 5X

Enhances adsorption of antigens while preserving structure.

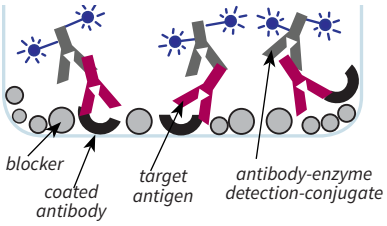
Antigen Coating Buffer, 5X is a protein-stabilizing solution that maximizes the adsorption of peptide and protein antigens onto polystyrene plates. During the plate coating process, the salt and pH buffering environment provided by Antigen Coating Buffer stabilizes the three-dimensional antigen structure, leading to the preservation of antigen associated epitopes. The 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 antigen. Therefore, use of Antigen Coating Buffer allows ELISA plates to be manufactured with high levels of precision and antigen epitope retention.

Antigens are typically coated onto ELISA plates at 0.2-10 µg/mL, using 50-200 µL of 1X coating solution per well. This range translates to approximately 1.1-4.4 mL of Antigen 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 x 110% = 31.7 mL needed.
- Antigen Coating Buffer, 5X: 31.7 mL / 5 = 6.3 mL.
- DiH_2O required: 31.7 mL - 6.3 mL = 25.4 mL diH_2O.
- Add 6.3 mL Antigen Coating Buffer, 5X to 25.4 mL diH_2O.
- Add the appropriate volume of antigen to the 1X coating solution to attain the target coating concentration.

As Antigen 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.

Antigen-Down 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.

ANTIGEN COATING BUFFER, 5X

Size	Catalog #
100 mL	#6247
500 mL	#6248
1 L	#6249
10 L	#6250

INSTRUCTIONS:

1. Calculate the amount of 1X coating solution needed. ELISA plates are typically coated with 50-200 µL per well.
2. Mix Antigen 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 Antigen Coating Buffer 1:5 by adding 1 part coating buffer to 4 parts diH_2O using.
4. Mix until crystals have dissolved.
5. Add the antigen to the 1X buffer at the target concentration. The optimal coating concentration typically ranges from 0.2-10 µg/mL.
6. Mix for 15 minutes.
7. Pipette the coating solution into each well of the microtiter plate, typically 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 300-400µL of blocking buffer (such as catalog #640, 64, or 643) 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).

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

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

STORAGE:
- 24 months at room temperature
- Product may 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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