NEUROMICS

ELISA Coating Buffer (CB1)-Prep and Protocol

Catalog #: KF17336 **Sizes: 100** ml, 500 ml, 1 L, 10 L

Storage: After dilution, the 1x buffer will suppress bacterial growth for up to 1 month at room temperature and 6 months at 2°-8°C. The concentrated 5x buffer will suppress bacterial growth up to 18 months at 2°-8°C.

Depending on the activity of the coated protein, plates may be stored at 2°-8°C (or even at room temperature for hardy proteins) for several months, or even years

Buffer Preparation

Simply dilute CB1 1:5 (for example, add 100mL CB1 to 400mL diH_20 for a final volume of 500mL) , add your protein antigen or antibody, let the solution stir for 15 minutes, and pipette onto the plate. As CB1 is concentrated, crystalline precipitates may form in the bottle, especially when refrigerated. If this happens, gently warm the buffer until all crystals are dissolved. Do not let it boil.

Coating Protocol

1. Dilute the 5x CB1 by adding 1 part buffer to 4 parts deionized water (100mL CB1 to 400 mL diH_2O, yielding a total volume of 500mL) and mix for 15 minutes.

2. Dilute your antigen or antibody into the coating buffer (coating concentration varies significantly from less than 0.1ug/mL to over 10 ug/mL).

3. Let the solution stir (10 - 15 minutes) and pipette onto the plate (coating volume generally ranges between 50-300 uL per well).

4. Once added to the plate, incubate the coating solution from 3 - 24 hours at RT protected from light (minimize evaporation by individually covering each plate with a plate sealer, wrapping a stack in plastic wrap, or placing plates in a humidified storage box, and cover).

5. After incubation, dump or aspirate the coating solution out of the wells.

6. Wash the plate 2 - 4 times with ICT's wash buffer.

7. Aspirate and pipette one of ICT's block buffers onto the plate at a higher volume than the coating solution (300-400uL per well).

8. Once added to the plate, incubate the block buffer from 3 - 24 hours at RT protected from light (minimize evaporation by individually covering each plate with a plate sealer, wrapping a stack in plastic wrap, or placing plates in a humidified storage box, and cover).

9. Aspirate the block buffer.

10. The assay can be run at this point, or the plate can be dried and packaged for later use.

11. Dry the plate by letting it sit on the bench top from 2 - 24 hours (but protected from light – loosely cover with aluminum foil), or dry in a drying oven from 2 - 24 hours at RT or warmer.

12. When dry, seal the plate in an air-tight foil pouch with a desiccant and store at RT or 2°-8°C protected from light.

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