

2/3/88 Doc.Stoll

### IgG Elispot

- Coat Immulon II or equivalent polystyrene, flat bottomed, 96 well ELISA plate with 2 ug/ml Goat-anti-mouse-IgG (SBA) in 0.1 M Carbonate buffer pH 9.6. Incubate overnight at room temperature (RT). Plates can then be frozen or used immediately.

-Flick out coating buffer, wash once with TBS and add 100 ul/well 3% BSA/TBS for one hour at RT.

-During this incubation prepare cells. For this experiment harvest the IgG secreting hybridomas, and wash twice with balanced salt solution. Resuspend at 2,000 cells/ 100 ul or 20,000 cells/ml in DMEM 10%FBS tissue culture media.

-Wash plates with balanced salt solution x 2 then add cells. Dilute cells on the plate (using tissue culture media) from neat (2000 cells) to 125 (5 dilutions). Cover plate and incubate at 37 C, 5-7% CO2 for 4 hours.

-Wash cells with TBS/0.5% Tween 20 until clear. Look at cells with the inverted microscope to assess whether washing is adequate.

-Add the second antibody, Goat-anti-mouse-IgG-Alkaline-phosphatase conjugate (1:1000) in 3% BSA/TBS/0.5% Tween 20. Incubate overnight in a refrigerator.

-Wash three times with TBS only. Develop with 0.6% agarose/BCIP/BCIP buffer. Count spots on low power with an inverted or dissection microscope.

Comments: I make TBS using TRIZMA 7.8 (sigma). All you need to do is weigh out 0.01 M TRIZMA (1.45 gm) and 0.9 M NaCl per liter of water. No pH adjustment is needed.