ELISPOT METHODS

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References:

1) Czerkinsky et. al. A solid -phase enzyme linked immunospot (ELISPOT) assay for enumeration of specific antibody-secreting cells. 1983. J. Immunol. Methods 65:109.

2) Sedgewick and Holt, A solid-phase immunoenzymatic technique for the enumeration of specific antibody secreting cells. 1983. J. Immunol. Methods. 57:301.

Materials.

ELISA plates coated with antigen. Most important is that the plates are flat bottomed ELISA plates. Tissue culture clusters do nto have adequate protein binding capacity. I use polyvinylchloride, flat bottomed plates from Dynatech or Co-star. There is great variability between plates from different manufacturers e.g. Fischer plates are terrible. The plates are coated with antigen according to your ELISA methods. ELISPOTs need to have a homogenous coating of antigen on the plate in order to detect AFC with high efficiency. For Lysozyme and KLH, I pretested known antisera by ELISA on plates coated with varying concentrations of antigen. Use the lowest concentration that results in a maximal OD.

Cell sources. Hybridomas, spleen cell suspensions or cells from Mishell-Dutton cultures can be used. I will give you methods for use in M-D cultures. Hybridomas are good positive controls to practice on. Use hybridomas that are in log phase growth and approximately 30% of counted cells should secrete and form AFC. Approximately 500-1000 hybridomas are maximal (without excess crowding). Using dilutions e.g. 1000/500/250/125 etc. will allow you to find the optimal concentrations of cells/well for a particular hybrid. Incubate cells on the plate for 3-4 hours for hybrids.(Cells vary in their rate of secretion of Ig)

Media. Cells are cultured in Iscoves DMEM with 3% BSA(Sigma), HEPES, glutamine, pyruvate, non-essential amino acids. I usually make up 500 ml at a time, refilter and store at 4 C.

Buffers:
I use Tris buffered saline (Tris .01 M, NaCL 0.14 M, pH 7.5) but PBS and Borate buffered saline can also be used.

Development buffer in agarose:
The major trick to this method is 1) a non-souble chromogen

BCIP is used and 2) development buffer is mixed with agarose to result in a 0.6% agarose solution which gels in the wells. Thus diffusion of precipitated chromogen is prevented allowing development of discrete spots where cells had secreted Ig.

Development buffer.

2-amino-2-methyl-1-propanol (AMP) from Sigma. A-9879
MgCl2- 6 H20 Sigma.
Triton-X 405 T-7253
5-Bromo-4-chloro-3-indolyl phosphate (5-BCIP) Sigma. B 8503
HCl 37% concentrate

Recipe for 2-AMP buffer 1M, pH 10.25, 1 liter.

AMP 95 ml
MgCl2 150 mg
Triton x 405 0.1 ml.

Add 95 ml of AMP to 800 ml of ddH20. Add MgCl2 and triton X. If water has calcium a precipitate will form. Stir for 20 minutes. Adjust pH down to 10.25 with the HCl. Do this in a chemical hood since chlorine gas is emitted. Store at 4 C. This is good for at least 6 months (probably longer).

BCIP is added to this buffer at 1 mg/ml prior to use.

Agarose:
Type I:Low EEO, Sigma A-6013.

Make a 3% agarose solution in ddH20 and aliqout into glass tubes 5ml/ tube. COver tubes with parafilm and store at 4 C. 3gms/100ml
1.5gms/50ml

Development buffer:

A 65 C water bath is needed for this step.

To calculate the amounts of agarose and BCIP buffer needed one part agarose is added to 4 parts of BCIP buffer. This results in a 0.6% agarose concetration in the final solution. Thus for a full 96 well plate 10 mls of development buffer will be needed. Thus 2ml of agarose and 8 ml of BCIP buffer will be needed. per 96 well plate

1) Add 8 mg of BCIP to 8 ml of AMP buffer. Heat at 65 C for 20 minutes.

2) Melt 3% agarose by boiling or microwave.

3) Add melted agarose (2ml) to BCIP buffer and incubate at 65 for 10 minutes.

4) Add development buffer in 100 ul aliquots to ELISPOT wells.