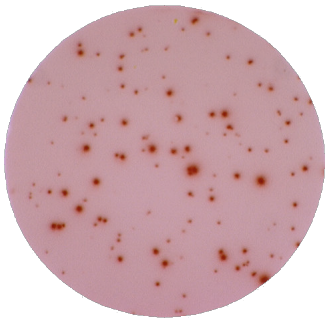
ELISPOT Plate Type Info

Deciding which type of plate to use for ELISPOT assays is a difficult task, due to the numerous types of plates available from different manufacturers, various ELISPOT protocols and, of course, the budget situation. CTL-Europe hereby provides you with information on the various recommended (and not recommended) types of plates to use with ImmunoSpot® equipment. In general, the decision whether sterile or non-sterile plates are used is influenced by the individual protocol, e.g. whether antibiotics may be added in the assay or not. All described plate types have 96-well formats, although ImmunoSpot® Analyzers are capable of reading 24- to 96-well plate formats. Please note that catalogue numbers might vary depending on your country.

PVDF Membrane Plates

CTL recommends in general using PVDF membrane plates for use in ELISPOT. Other membranes, such as mixed cellulose ester, use electrostatic interaction for the binding of antibodies. PVDF (polyvinylidene difluoride) uses hydrophobic interactions. The hydrophobic interactions of amino acids such as phenylalanine or leucine as present in the antibody is stronger than the electrostatic interaction, and thus one needs in general lower antibody concentrations to coat the plates. Furthermore, the antibodies have a higher density on the membrane surface, and thus produce in general spots that are more precise. PVDF membrane plates are available for example from Millipore:

- Catalogue No S2EM004M99: White, sterile 0.45µm Immobilon-P hydrophobic PVDF membrane; MultiScreen 96-well filtration and assay plate
  These plates are the ones strongly recommended by CTL
- Catalogue No ELIIP10SSP: White, sterile MultiSceen 96-well plate without underdrain in single-well tray
- Catalogue No MAIPS4510: Clear, sterile 0.45µm Immobilon-Phydrophobic PVDF membrane; MultiScreen 96-well filtration and assay plate. Please note that light reflections in clear plates are inevitable upon scanning the plates. Therefore, the use of white or opaque plates is to be preferred.



Peel-Off Plates (Nylon Membrane):

Some plates do not have individual membranes in each well, but feature a square membrane covering the whole plate bottom. These plates can be used in the ImmunoScan™ system when peeled off. If not peeled off, they can be used in CTLs analyzer units. Plates are available from Nunc, and feature a nylon membrane. Nylon is a generic name for a family of long-chain polyamide thermoplastics, which have recurring amide groups [-CO-NH-] as an integral part of the main polymer chain. Nylons are synthesized from intermediates such as dicarboxylic acids, diamines, amino acids and lactams, and are identified by numbers denoting the number of carbon atoms in

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the polymer chain derived from specific constituents, those from the diamine being given first. The second number, if used, denotes the number of carbon atoms derived from a diacid. Nunc Silent Screen plates feature a nylon 6/6 membrane, in general made by condensing hexamethylenediamine [$H_2N(CH_2)_6NH_2$] with adipic acid [$COOH(CH_2)_4COOH$]. Both clear and white, sterile and non-sterile plates with and without lid are available with Biodyne® A and B membranes:

Catalogue No 255998: 0.45µm white sterile Biodyne®A
Catalogue No 255997: 0.45µm white sterile Biodyne®B

Cellulose Nitrate Membrane Plates:

e.g. UniFilter white polystyrene microplates by Whatman. This plate features a 0.45µm cellulose nitrate membrane, with a capacity of 350µl. Catalogue No 7700-3307. Cellulose nitrate is made by treating fibrous cellulosic materials with a mixture of nitric [HNO_3] and sulfuric acids.

Other Mixed Cellulose Ester Membrane Plates:

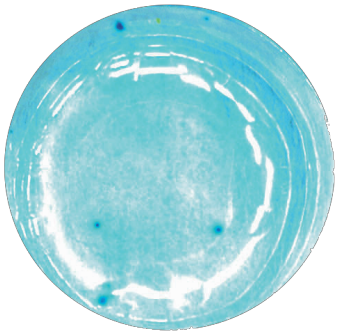
The binding of antibodies on mixed cellulose ester membranes is based upon electrostatic interactions. Plates are e.g. available from Millipore, Catalogue No ELIHP10SSP, White, sterile MultiSceen 96-well plate without underdrain in single-well tray.

Low Volume Plates

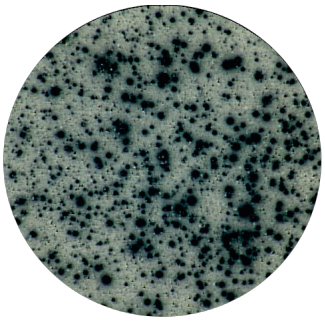
If cell numbers are critical, low volume plates can be used: CTL strongly recommends using UniFilter white polystyrene microplates by Whatman, 0.45µm cellulose nitrate membrane, with a capacity of 50µl. Catalogue No 7700-0009.

Agarose Plates

Unlike ELISPOT plates with membranes, in the agarose gel overlay method the BCIP substrate is added in a gel overlay. Agarose gel overlay plates are for example available at Cellsciences. However, as tested by CTL scientists, the spots in Agarose overlay plates do tend to fade quickly, and therefore need to be scanned ideally the same day. In addition, the Agarose overlay creates strong light reflections in the well, and makes the analysis process more tedious for the operator.



Clear Bottom Membrane Less Plates



In addition to plates with membranes and agarose overlay plates, there are clear-bottom plates available where the antibody is coupled directly to the bottom of the plate. The resulting spots appear white or golden. These images can be easily captured by ImmunoSpot® Analyzers, and analyzed by color inversion of the image.

www.cellsciences.com
www.millipore.com
www.nuncbrand.com
www.whatman.com

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