



ELISPOT Plate Type Information

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 the ImmunoSpot® S4 Analyzer is 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 (view Image 1)**
- Catalogue No ELIIP10SSP: White, sterile MultiScreen 96-well plate without underdrain in single-well tray
- Catalogue No MAIPS4510: Clear, sterile 0.45µm Immobilon-P hydrophobic PVDF membrane; MultiScreen 96-well filtration and assay plate. Please note that light reflections in clear plates are inevitable upon scanning the plates, which leads to uneven illumination in the single wells. Therefore, the use of white or opaque plates is to be preferred.

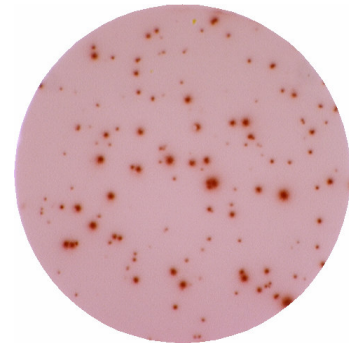


Image 1

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 easily be used in the ImmunoScan™ system when peeled off. If not peeled off, they can be used in S4 and S4 UV 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 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 [H₂N(CH₂)₆NH₂] with adipic acid [COOH(CH₂)₄COOH]. Both clear and white, sterile and non-sterile plates with and without lid are available with Biodyne® A and B membranes:

Cat No 255998 0.45µm white sterile Biodyne®A
 Cat 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 [HNO₃] 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 (please view Image 2).

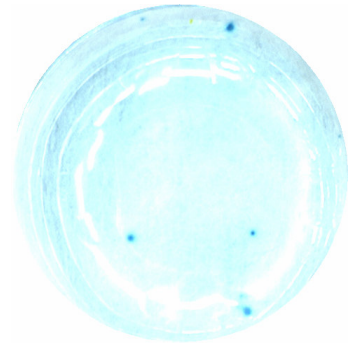
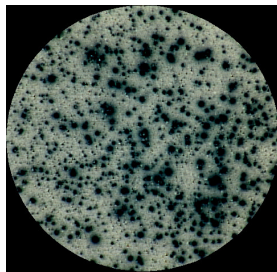


Image 2

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 the ImmunoSpot® S4 and S4 UV Analyzers, and analyzed by color inversion of the image (Please view Image 3)

Image 3

Summary:

Immuno-Spot® Products	PVDF white	PVDF clear	Peelable Nylon white	Peelable Nylon clear	Cellulose Nitrate	Other Mixed Cellulose Ester	Low-Volume	Agarose	Clear-Bottom Membrane-less
Immuno-Scan™	If punched	If punched			If punched	If punched			
S4 Analyzer									
S4 UV Analyzer									

Recommended Analyzable Not Analyzable

www.cellsciences.com
www.millipore.com
www.nuncbrand.com/index.asp
www.whatman.com

