

# PeliSPOT™

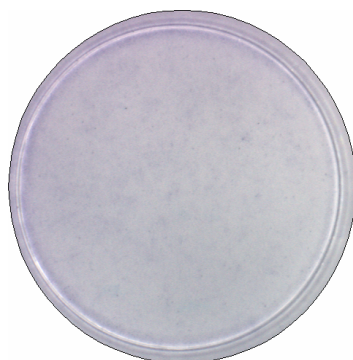
## Human Perforin

<b>Order number</b>	M9437
<b>Specificity</b>	The monoclonal antibodies of this pair recognize native human Perforin
<b>Intended use</b>	<b>For research use only</b>
<b>Application</b>	<p>CD8<sup>+</sup> cytotoxic T lymphocytes (CTL) are major players in immune surveillance for elimination of virus infected cells, certain tumor cells, and MHC class I incompatible cells. To achieve their different cytotoxic functions, CTLs employ mainly two mechanisms: the Fas/Fas-ligand interaction and the system of lytic granules of granzymes and the pore-forming protein Perforin. After a CD8<sup>+</sup> CTL has recognized a target cell by interaction between its T-cell receptor and the antigenic peptide bound to the MHC class I-complex, Perforin is released and the immunological synapse is formed, resulting in the collapse of the target membrane. Water, ions and proteases, like granzymes B, then enter the target cell and initiate the apoptotic cascade.</p> <p>Perforin is a 70- kDa protein which is produced and released by CTLs and Natural Killer cells after antigen specific stimulation with peptides or polyclonal stimulation with PMA combined with ionomycin.</p> <p>The human Perforin PeliSPOT™ assay has the capacity to identify antigen specific T- or B-cells at single cell level without long-term culture or flow cytometric analysis with tetramer staining. The Perforin PeliSPOT™ assay exhibits a high level sensitivity that permits detection of a few CMV- or EBV- peptide responding cells due to the low background with 100,000 PBMCs.</p> <p>This PeliSPOT™ human Perforin pair has been developed for reproducible and specific enumeration of human Perforin secreting cells.</p>
<b>Assay procedure</b>	See PeliSPOT™ product information
<b>Storage and stability</b>	As indicated on the box label
<b>Cell incubation</b>	Optimal conditions should be determined by the researcher. A typical incubation period is 48 hours.
<b>Positive secretion control</b>	Polyclonal cell activation with PMA (1 ng/ml) + ionomycin 10 uM is recommended.
<b>Spot counting</b>	Enumeration of spots is preferably done with the A.EL.VIS spot analysers, Eli.Scan or Eli.Expert.

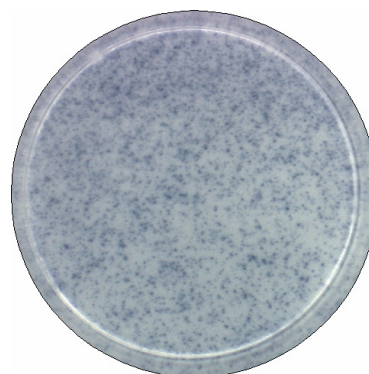
Recommended parameter settings for software version V3.3 and up:

ROI	= 70%	Invert	= off
Brightness	= 65%	Slope	= N
MinSize	= 3	Development	= N
MaxSize	= 500	Separation	= 50
Minintensity	= 10	Pollution	= On
MinCircularity	= 100	Overdevelop	= On

**Typical results after 48 hours incubation**



100,000 PMNC cells per well,  
non-stimulated



100,000 cells per well,  
PMA/iono stimulated