

Contents

1 Overview	1
1.1 Historic Overview	1
1.2 Principles of Elispot	2
1.3 Modifications	4
1.3.1 Simplifications	5
1.3.1.1 Coating Step	5
1.3.1.2 Detection Step	6
1.3.2 Adaptations	6
1.3.2.1 Detection of Specific Antibodies.....	6
1.3.2.1 Detection of Multiple Analytes in One Well.....	7
1.4 Summary	10
2 Important Assay Details	11
3 Reagents and Materials	13
3.1 Elispot Plates.....	13
3.2 Antibody Choices.....	17
3.3 Enzymatic Conjugates and Substrates	17
3.4 Fluorescent Dyes.....	19
3.5 Culture Media	19
3.6 Blocking Buffers	21
3.7 Washing Buffers.....	21
3.8 Important Laboratory Supplies	22
3.8.1 Pipettes.....	22
3.8.2 Filters	23
3.8.3 Other Supplies.....	23
3.9 Automated Elispot Readers.....	23
4 Sample Preparation	25
4.1 Overview	25
4.2 Whole Blood and PBMC	26
4.2.1 Blood Draw and Anticoagulants.....	26

- 4.2.2 PBMC Isolation 26
- 4.2.3 Time Frame Between Blood Draw and PBMC Isolation 28
- 4.3 Immune Cells Obtained from Tissue 31
- 4.4 Freezing of Cells 31
- 4.5 Thawing of Cells 33
- 4.6 Cell Counting and Viability 34
- 4.7 Overnight Resting 36
- 4.8 Isolation of Subpopulations of Cells 39
- 4.9 Expansion of Cells 40
- 5 Planning the Experiment..... 43**
 - 5.1 Controls..... 43
 - 5.1.1 Negative Controls..... 43
 - 5.1.2 Positive Controls 44
 - 5.1.3 External Controls 46
 - 5.2 Plate Layout 46
 - 5.3 Timing 47
- 6 The Elispot Assay 49**
 - 6.1 Coating 49
 - 6.2 Blocking 50
 - 6.3 Effector Cell Preparation 51
 - 6.4 Stimulant Preparation..... 52
 - 6.4.1 Peptides and Peptide Pools 52
 - 6.4.2 Proteins and Lysate 54
 - 6.4.3 Antigen Presenters 54
 - 6.4.4 Control Stimulants 55
 - 6.5 Reference Samples..... 56
 - 6.6 Plating Out Cells and Stimulants 56
 - 6.7 Incubation 59
 - 6.8 Removal of Cells..... 59
 - 6.9 Spot Development..... 60
 - 6.10 Enumeration of Spots..... 61
- 7 Data Analysis 67**
- 8 Quality Control 73**
- 9 Reporting Guidelines 79**
- 10 Troubleshooting..... 81**
- References..... 83**
- Index..... 91**



<http://www.springer.com/978-3-319-45293-7>

Elispot for Rookies (and Experts Too)

Janetzki, S.

2016, VIII, 93 p. 39 illus., 32 illus. in color., Hardcover

ISBN: 978-3-319-45293-7