

8. Appendix

8.1. Abbreviations

APC antigen presenting cells

BIP bactericidal/permeability increasing protein

CD Cluster of differentiation

CRF corticotropin releasing factor

DIC disseminated intravascular coagulation

DD death domain

ELISA enzyme-linked immunosorbent assay

ERK extracellular signal-regulated kinase

fMLP f-Met-Leu-Phe peptide

GPI glycosylphosphatidylinositol

HDL high density lipoprotein

ICAM intercellular adhesion molecule

ICE interleukin 1 converting enzyme

IFN interferon

IL Interleukin

IL-1ra IL-1 receptor antagonist

IL-1RI and II Interleukin-1 receptor 1 and 2

IRAK IL-1-receptor-associated kinase

JAK Janus kinase

JNK c-Jun N-terminal kinase

KDO 3-keto-deoxy-D-manno-octulosonic acid

LAL Limulus amoebocyte lysate

LBP LPS binding protein

LFA leukocyte functional antigen

LPS lipopolysaccharide

LTA lipoteichoic acid

MALP mycoplasmal macrophage-activating lipopeptide

MAPK mitogen-activated protein kinase

MyD myeloid differentiation factor 88

NIBSC national institute for biological standards and controls

NFκB nuclear factor κB

NLS nuclear localization signal
OVLT organum vasculosum laminae terminalis
PAMP pathogen-associated molecular pattern
PMN polymorphonuclear cells
RAIDD RIP associated ICH-1/CED-3 homologous protein with death domain
RNA ribonucleic acid
RIP receptor interacting protein
STAT signal transducer and activator of transcription
TIR TOLL/interleukin 1 receptor
TLR toll-like receptor
TNF Tumor Necrosis Factor
TRADD TNF receptor associated death domain
TRAF TNF-receptor associated factor
USP United States Pharmacopoe
WEHI Walter and Elisabeth Hall Institute
WHO world health organisation

8.2. Tables

Table 1: human IL-1 β ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (1 μ g/ml) in Coating Buffer 50 μ l/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight at 4°C		
Blocking	PBS-BSA, 3%, 200 μ l/well	PBS-BSA:
Leave 2 h at room temperature		
Washing step: 3x		
Addition of antigen	Supernatants, 50 μ l/well Standard curve: recombinant IL-1 β , in PBS-BSA 5 ng/ml-6.8 pg/ml	Recombinant human IL-1 β in PBS-BSA
Addition of detection antibody	Detection antibody, (0.15 μ g/ml) in PBS-BSA 50 μ l/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 μ l/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 μ l/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 μ l/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 2: human IL-6 ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (1.25 µg/ml) in Coating Buffer 50 µl/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight at 4°C		
Blocking	PBS-BSA, 3%, 200µl/well	PBS-BSA
Leave 2 h at room temperature		
Washing step: 3x		
Addition of antigen	Supernatants, 100 µl/well Standard curve: recombinant IL-6 in PBS-BSA 3 ng/ml-4.1 pg/ml	Recombinant human IL-6, in PBS-BSA
Leave 3 hours at room temperature		
Wash 4x		Washing Buffer
Addition of detection antibody	Detection antibody, (30 ng/ml) in PBS-BSA 100 µl/well	
Leave 60 minutes at room temperature		
Washing step (6x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 µl/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 µl/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 µl/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 3: human TNF- α ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (2.5 μ g/ml) in Coating Buffer 50 μ l/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight at 4°C		
Blocking	PBS-BSA, 3%, 200 μ l/well	PBS-BSA
Leave 2 h at room temperature		
Washing step: 3x		
Addition of antigen	Supernatants, 50 μ l/well Standard curve: recombinant TNF- α , in PBS-BSA 2 ng/ml- 4.3 pg/ml	Recombinant human TNF- α , in PBS-BSA
Addition of detection antibody	Detection antibody, (0.25 μ g/ml) in PBS-BSA 50 μ l/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 μ l/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 μ l/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 μ l/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 4: human IL-8 ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (0.5 µg/ml) in Coating Buffer 50 µl/well	Coating Buffer (NaHCO ₃) pH 8.2
Overnight at 4°C		
Blocking	PBS-BSA, 3%, 200µl/well	PBS-BSA
Leave 2 h at room temperature		
Washing step: 2x		
Addition of antigen	Supernatants, undiluted or diluted 1:10, 50 µl/well Standard curve: recombinant IL-8, in PBS-BSA, 5 ng/ml-6.8 pg/ml	Recombinant human Il-8, in PBS-BSA
Leave 1 h at room temperature		
Washing step: 4x		
Addition of detection antibody	Detection antibody, (30 ng/ml) in PBS-BSA 50 µl/well	
Leave 90 minutes at room temperature		
Washing step (8x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 µl/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 µl/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 µl/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 5: rabbit IL-1 β ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (0.2 μ g/ml) in Coating Buffer 50 μ l/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight at 4°C		
Blocking	PBS-BSA, 3%, 200 μ l/well	PBS-BSA
Leave 2 h at room temperature		
Washing step: 3x		
Addition of antigen	Supernatants, 50 μ l/well Standard curve: recombinant rabbit IL-1 β , in PBS-BSA 5 ng/ml-6.8 pg/ml	Recombinant rabbit IL-1 β PBS-BSA
Addition of detection antibody	Detection antibody, (0.2 μ g/ml) in PBS-BSA 50 μ l/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 μ l/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 μ l/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 μ l/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 6: rabbit IL-6 ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (1 µg/ml) in Coating Buffer, 50 µl/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight		
Blocking	PBS-BSA, 3%, 200µl/well	PBS-BSA
Leave 2 h at room temperature		
Washing step: 3x		Washing Buffer
Addition of antigen	Supernatants, undiluted, 50µl/well Standard curve: recombinant IL-6, in PBS-BSA 5 ng/ml-6.8 pg/ml	Recombinant IL-6 in PBS-BSA
Leave overnight at 4°C		
Wash 4x		Washing Buffer
Addition of detection antibody	Detection antibody, (1 µg/ml) in PBS-BSA 50 µl/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 µl/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 µl/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 µl/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 7: rabbit TNF- α ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (1 μ g/ml) in Coating Buffer, 50 μ l/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight at 4°C		
Blocking	PBS-BSA, 3%, 200 μ l/well	PBS-BSA:
Leave 2 h at room temperature		
Washing step: 3x		
Addition of antigen	Supernatants, undiluted, 50 μ l/well Standard curve: recombinant TNF- α , in PBS-BSA 5 ng/ml-6.7 pg/ml	Recombinant rabbit TNF- α , in PBS-BSA
Leave overnight		
Wash 4x		Washing Buffer
Addition of detection antibody	Detection antibody, (0.5 μ g/ml) in PBS-BSA 50 μ l/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 μ l/well	POD, PBS-BSA
Room temperature		
Washing step: 8x		Washing Buffer
Addition of TMB	100 μ l/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 μ l/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 8: rabbit IL-8 ELISA

STEP	REAGENTS	BUFFERS
Coating	Capture antibody, (1:2000) in Coating Buffer, 50 µl/well	Coating Buffer (NaHCO ₃) pH 8.2
Leave overnight		
Blocking	PBS-BSA, 3%, 200µl/well	PBS-BSA:
2 h RT		
Washing step: 3x		
Addition of antigen	Supernatants, diluted (1:10-1:50), 50 µl/well Standard curve: recombinant IL-8, in PBS-BSA, 5 ng/ml-6.7 pg/ml	Recombinant rabbit IL-8 in PBS-BSA
90 minutes at RT		
Wash 4x		Washing Buffer
Addition of detection antibody	Detection antibody, (1:1000) in PBS-BSA 50 µl/well	
Leave 90 minutes at room temperature		
Washing step (4x)		Washing Buffer
Addition of POD	POD, 50 ng/ml in PBS-BSA, 100 µl/well	POD, PBS-BSA
Leave at room temperature, 30 min		
Washing step: 8x		Washing Buffer
Addition of TMB	100 µl/well	TMB
Development of color reaction		
Stop of color reaction	H ₂ SO ₄ , 50 µl/well	H ₂ SO ₄
Reading in the ELISA reader		

Table 9: Scheme for the WEHI assay

Standard (pg/ml)	Samples (Dilution factor)	TNF	Control
100	10		Control
25	40		
6.25	160		
1.56	640		
0.39	2560		
0.098	10240		Lysis Control
0.024	40960		
0.006	163840		