Appendix A

Chemical Reagents

1. Reagent for DPPH radical scavenging assay

- **Working DPPH (6×10^{-5} M)**

DPPH	2.4	mg
Absolute ethanol	100	ml

(Freshly prepared, wrapped foil, stored at 4°C)

2. Reagents for determination NO

- **Griess reagent**

Sulfanilamide	1.0	g
N-(1-Naphthyl)ethylenediamine dihydrochloride	0.1	g
Phosphoric acid	2.5	g

Adjust volume with MQ water to 100 ml

(Stored at 4°C)

- **MTT solution (5 mg/ml)**

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide or Thiazolyl blue tetrazolium bromide	200	mg
PBS	40	ml

(Wrapped foil and stored at 4°C)

- **0.04 M HCl in Isopropanol**

HCl	0.83	ml

Adjust volume with Isopropanol to 250 ml

(Stored at room temperature)

3. Reagents for determination TNF-α

- **Wash buffer solution**

Wash buffer	25	ml
Distilled water	600	ml

(Stored at 4°C)

- **Substrate solution**

Color reagent A and B should be mixed together in equal volumes

(Freshly prepared, wrapped foil)

4. Reagents for determination β-hexosaminidase

- **Siraganian buffer (Buffer A) (pH 7.2)**

			g/l	
NaCl	119	mM	6.954	g
KCl	5	mM	0.373	g
Glucose	5.6	mM	1.009	g
$MgCl_2.6H_2O$	0.4	mM	0.081	g
$CaCl_2.2H_2O$	1	mM	0.147	g
PIPES	25	mM	8.396	g
BSA	0.1	%	1.000	g
Distilled water			900	ml

Adjust pH 7.2 by 0.1 N NaOH, then adjust volume to 1,000 ml

(Stored at room temperature)

- **0.1 M Citric buffer (pH 4.5)**

			g/500 ml	
Citric acid monohydrate	0.1	M	10.51	g
Trisodium citrate dehydrate	0.1	M	14.71	g

(Stored at room temperature)

- **0.1 M Na_2CO_3 buffer (pH 10.0)**

			g/500 ml	
Na_2CO_3	0.1	M	5.3	g
$NaHCO_3$	0.1	M	4.2	g

(Stored at room temperature)

- **DNP-IgE solution**
  - Stock DNP-IgE solution (50 μg/ml)

DNP-IgE	0.5	ml
PBS	9.5	ml

(Aliquot, stored at -20°C)

  - Working DNP-IgE solution

Stock DNP-IgE solution	100	μl
Media MEM	900	μl

(Freshly prepared)

- **DNP-BSA solution (0.1 mg/ml)**

DNP-BSA	10	mg
Buffer A	100	ml

(Aliquot, stored at -20°C)

5. Reagents for cell culture

- **FBS (inactivated)**

Slowly thaw the frozen FBS, heat inactivate (56°C, 30 min)

(Aliquot, stored at -20°C)

- **RPMI 1640 (incomplete media)**

RPMI 1640 1X with L-glutamine	1	pack
$NaHCO_3$	2.0	g

Adjust volume with sterile water to 1,000 ml

Adjust pH 7.00-7.05 by 1 N NaOH or 1 N HCl

(Filtered sterile at a pore size of 0.2 μM, stored at 4°C)

- **RPMI 1640 (complete media)**

RPMI 1640 (incomplete media)	400	ml
10% FBS	40	ml
1% Penicillin-Streptomycin	4	ml

  (Stored at 4°C)

- **MEM (incomplete media)**

MEM 1X with Earle's salts, L-glutamine	1	pack
$NaHCO_3$	2.2	g

  Adjust volume with sterile water to 1,000 ml

  Adjust pH 7.00-7.05 by 1 N NaOH or 1 N HCl

  (Filtered sterile at a pore size of 0.2 μM, stored at 4°C)

- **MEM (complete media)**

MEM (incomplete media)	400	ml
10% FBS	40	ml
1% Penicillin-Streptomycin	4	ml

  (Stored at 4°C)

- **PBS**

PBS	1	tablet
MQ water	100	ml

  (Autoclave 121°C, 15 min, stored at 4°C)

- **Penicillin-Streptomycin**

  Slowly thaw the frozen P/S in water bath at 37°C till complete thaw

  (Aliquot, stored at -20°C)

- **Trypsin-EDTA**

  Slowly thaw the frozen trypsin in water bath at 37°C till complete thaw

  (Aliquot, stored at -20°C)

6. Assay procedure of Quantikine mouse TNF-α ELISA test kit

Bring all reagents to room temperature

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Prepare reagents and samples

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Add 50 µl Assay Diluent RD1W to each well

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Add 50 µl Standard, Control, or sample to each well

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Gentle mix

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Cover the plate and incubate for 2 h at room temperature

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Aspirate and wash each well five times with Wash buffer (400 µl)

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Add 100 µl mouse TNF-α Conjugate to each well

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Cover the plate and incubate 2 h at room temperature

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Aspirate and wash each well five times with Wash buffer (400 µl)

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Add 100 µl Substrate solution to each well, incubate 30 min at room temperature, protect from light

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Add 100 µl Stop solution to each well

↓

Gentle mix

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Read optical density at 450 nm of each well within 30 min