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APPENDIX

1. Complete RPMI 1640 100 ml

RPMI 1640	90%	
FBS	10%	
Penicillin	100	U/ml
Streptomycin	0.4	mg/ml
Sodium pyruvate	1%	
HEPES	1%	

2. Complete DMEM 100 ml

DMEM	90%	
FBS	10%	
Penicillin	100	U/ml
Streptomycin	0.4	mg/ml
Sodium pyruvate	1%	
HEPES	1%	

3. Complete MEM 100 ml

MEM	90%	
FBS	10%	
Penicillin	100	U/ml
Streptomycin	0.4	mg/ml
HEPES	1%	

4. Freezing media 10 ml

Complete media	90%
DMSO	10%

5. DEPC water for RNA work 100 ml

HPLC water (100 ml) was added into a clean bottle, and 10 µl of DEPC (0.01% v/v) was added into the water. Then, the bottle was swirled and incubated overnight at room temperature. Next, the DEPC water was autoclaved at 121°C and pressure 15 psi for 15 min.

6. FBS inactivation

Before using FBS, FBS must be inactivated at 56°C for 30 minutes using water bath.

7. 100 mM DAPT

DAPT (5 mg) was dissolved completely in 115.7 µl of DMSO and aliquoted into 10 µl in 1.5 ml microcentrifuge tubes and kept at -80°C.

8. 50×TAE buffer for agarose gel electrophoresis 200 ml

Trisma base	48.4	g
Glacial acetic acid	11.42	ml
0.5 M EDTA	20	ml

Adjusted pH to 8.0 and volume to 200 ml using deionized water and autoclaved at 121°C and pressure 15 psi for 15 min

8.1 2% agarose gel preparation

Agarose gel	2%	
1×TAE	20	ml

8.2 Running buffer for agarose gel electrophoresis

50×TAE was diluted to a final concentration of 0.5× in 400 ml of deionized water.

9. Lauria-Bertani (LB) broth and agar plate 1000 ml

9.1 LB broth preparation

Bacto tryptone	10	g
Yeast extracts	5	g
NaCl	10	g

Adjusted pH to 7.4 and volume to 1000 ml using deionized water

9.2 LB agar preparation

After LB broth preparation, 1.5% of agar was added to the broth. Next, the LB broth and agar were autoclaved at 121˚C and pressure 15 psi for 15 min.

9.3 LB agar containing 50 µg/ml ampicillin

After sterilization, the LB agar was warmed to approximately 50°C using water bath. Next, 50 µg/ml ampicillin was added to the warmed agar, and the agar was mixed well and poured plate.

10. SDS-polyacrylamide gel preparation

10.1 8% separating gel 8 ml

Sterile water	4.236	ml
40% Acrylamide and Bis-acrylamide solution	1.6	ml
1.5 M Tris-HCl pH 8.8	2	ml
10% SDS	0.08	ml
10% APS	0.08	ml
TEMED	0.004	ml

10.2 5% stacking gel 2 ml

Sterile water	1.204	ml
40% Acrylamide and Bis-acrylamide solution	0.25	ml
1 M Tris-HCl pH 6.8	0.504	ml
10% SDS	0.02	ml
10% APS	0.02	ml
TEMED	0.002	ml

11. 2×Laemmli buffer (SDS-dye) 10 ml

1 M Tris-HCl pH 6.8	1	ml (final concentration 100 mM)
10% SDS	4	ml (4% v/v)
99.5% glycerol	2.01	ml (20% v/v)
HPLC water	2.989	ml
Bromphenol blue	0.001	g

12. RIPA buffer for protein extraction 10 ml

50 mM Tris-HCl, pH 7.4	1	ml
150 mM sodium chloride	1.5	ml
1.0%NP-40	100	µl
0.5% sodium deoxycholate	1	ml
0.1% sodium dodecyl sulfate	100	µl
Adjusted volume to 10 ml using deionized water		

13. PBST (washing buffer for Western blot)

1×PBS	500	ml
Tween20	0.05%	

14. Blocking solution for Western blot

PBST	200	ml
Non-fat dry milk	3%	

15. 1×PBS pH 7.4

NaCl	8	g
KCl	0.2	g
Na_2HPO_4	1.44	g
KH_2PO_4	0.24	g
Deionized water	1000	ml

Autoclaved at 121°C and pressure 15 psi for 15 minutes

16. 5×running buffer for Western blot

Trisma base	15.1	g
Glycine	94	g
SDS	5	g
Deionized water	1000	ml

17. Transfer buffer for Western blot

Trisma base	5.08	g
Glycine	2.9	g
SDS	0.37	g
Deionized water	800	ml
Absolute methanol	200	ml

18. ECL substrate of HRP

Coumaric acid (90mM) was dissolved in DMSO in total volume 10 ml. Then, the solution aliquots were kept at –20°C.

Luminol (250 mM) was also dissolved in DMSO in total volume 10 ml. Then, the solution aliquots were kept at –20°C.

18.1 Solution A

100 mM Tris-HCl pH 8.5 (stored at 4°C)	4	ml
90 mM coumaric acid	17.6	µl
250 mM luminol	40	µl

18.2 Solution B

100 mM Tris-HCl pH 8.5 (stored at 4°C)	4	ml
30% H_2O_2	2.4	µl

19. Film developer and fixer

Film developer and fixer were diluted in tap water at dilution 1 : 4 in total volume 500 ml.

20. Ampicillin, penicillin and streptomycin

Ampicillin, streptomycin and G418 solution were prepared at final concentration 50 mg/ml, and penicillin was prepared at final concentration 10^6 U/ml by diluting in sterile deionized water. Then, the solutions were filtered by using 0.22 μm syringe filter. The solution aliquots were kept at –20°C.

21. 4% Paraformaldehyde

Paraformaldehyde (4g) was dissolved in 100 ml of PBS. After addition of a few drops of 1 N NaOH, the solution was heated at 65°C in a chemical hood. Then, the solution was cooled to room temperature and, the pH was adjusted to 7.4.

22. MTT 5 mg/ml in PBS

MTT	50	mg
Sterile PBS	10	ml

MTT was dissolved in sterile PBS and filtered through a 0.22 μm acrodisc syringe filter. Aliquoted into 1 ml in 1.5 ml microcentrifuge tubes and kept at 4°C.

23. 0.04 N HCL in Isopropanol

Isopropanol	80	ml
HCL	0.331	ml

Adjusted volume to 100 ml using isopropanol in volume metric flask.

24. Propidium iodide

Propidium iodide was dissolved in sterile PBS at 1 mg/ml. Solutions were stable if stored at 0-4°C in the dark.

25. 2% crystal violet in methanol

Crystal violet	2	g
Methanol (HPLC grade)	100	ml

Filtered and stored in the dark at room temperature.

Biography

Yanin Kuncharin was born in Bangkok, Thailand on September 30, 1983. After graduation with the Bachelor's degree of Science from the Department of Microbiology, Faculty of Science at Chulalongkorn University in 2004, she worked in the Virology Unit, Department of Microbiology, Faculty of Medicine at Chulalongkorn University in 2004-2006. She subsequently enrolled in the Master Program of Science in Medical Microbiology at Chulalongkorn University in 2007.

Academic presentation

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