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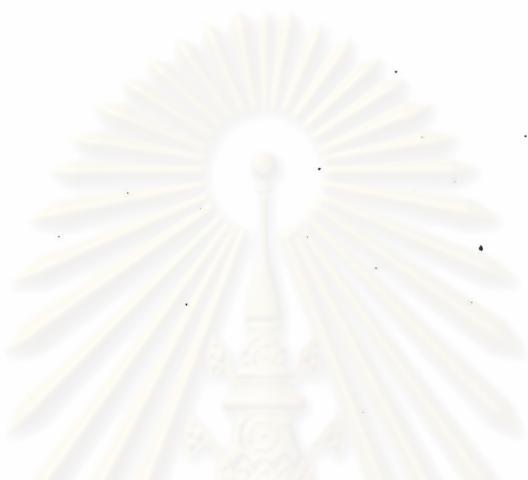
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APPENDIX

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

APPENDIX

Buffer and Reagent for Immunohistochemistry

1. Buffer solution preparation

0.2 M Na-KPB pH 7.4

Na ₂ HPO ₄	=	0.162 M	=	22.98 gm/H ₂ O
KH ₂ PO ₄	=	0.038 M	=	5.16 gm/H ₂ O

0.1 M Na-KPB pH 7.4

0.2 M Na-KPB	=	1000 ml
H ₂ O	=	1000 ml

0.1 M Na-KPB (saline) pH 7.4

0.1 M Na-KPB	=	100 ml
NaCl	=	0.8 gm

0.05 M Tris-HCl pH 7.4

Dissolve Tris	6.1 gm in H ₂ O	=	800 ml
Add 5 N HCl until pH 7.6			
Add H ₂ O until		=	1000 ml

4% Paraformaldehyde in 0.1 M Na-KPB pH 7.4: 100 ml

Paraformaldehyde	=	4 gm
Add H ₂ O	=	50 ml in (hood)
Heat at 60°C and stir		
Add 1 N NaOH until clear in color		
Add 0.2 M Na-KPB	=	50 ml

PBS-A (0.3% Triton X-100, 1% BSA)

PBS	=	100	ml
BSA	=	1	gm
Triton X-100	=	0.3	ml

PBS-B (0.1% Triton X-100, 0.25% BSA)

PBS	=	100	ml
BSA	=	0.25	gm
Triton X-100	=	0.1	ml

2. Stock DAB in Tris

Dissolve DAB 60 mg / Tris 12 ml

Filter

Pipette 1 ml into polypropylene tube (about 10 tubes then
freeze in refrigerator).

3. Working DAB (0.05% DAB in Tris) 0.01% H₂O₂

Stock DAB 1 tube + Tris 9 ml

Add 3.3 μl of 30% H₂O₂

Sample and Reagent Preparation for Nitrate and Nitrite Assay

Sample Preparation

All samples require at least a 2-fold dilution into Reaction Buffer (1x). After dilution, samples must be ultrafiltered through a 10,000 Molecular Weight cutoff filter to eliminate proteins.

Reagent Preparation

1. *Reaction buffer concentration (1x)*

Dilute 30 ml of reaction buffer concentration (10x) into distilled water to prepare 300 ml of reaction buffer (1x).

2. *NADH reagent*

Reconstitute, the NADH with 1 ml distilled water. Allow the NADH to sit for 3 minutes with gentle agitation prior to use. (Keep tightly capped on ice for the duration of the assay).

Dilution, immediately before use, dilute 900 μ l of NADH with 1.8 ml of distilled water (Keep on ice for the duration of the assay).

3. *Nitrate reductase*

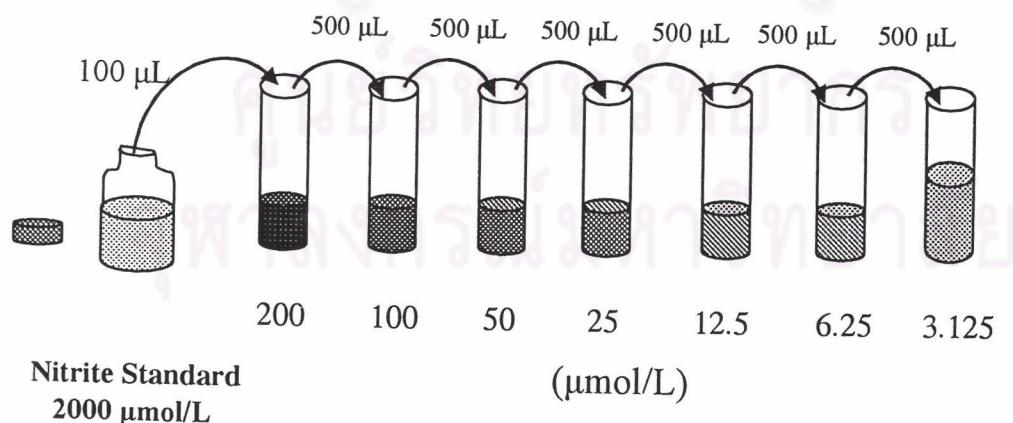
Reconstitute, the nitrate reductase with 1 ml nitrate reductase storage buffer. Vertex vigorously and allow to sit for 15 minutes at room temperature. Vertex again and allow to sit for an additional 15 minutes at room temperature. Vertex again. (Keep on ice for the duration of the assay).

Dilution, immediately before use, dilute the nitrate reductase using the following equation. Determine the number of wells to be used (all samples and standards should be assayed in duplicate).

- a. Nitrate reductase (μl) = (#wells + 2) \times 10 μl
- b. Reaction buffer (μl) = volume from step a \times 1.5
- c. Add volumes from steps a and b to a tube, vertex.
- d. Place on ice and use within 15 minutes of dilution.

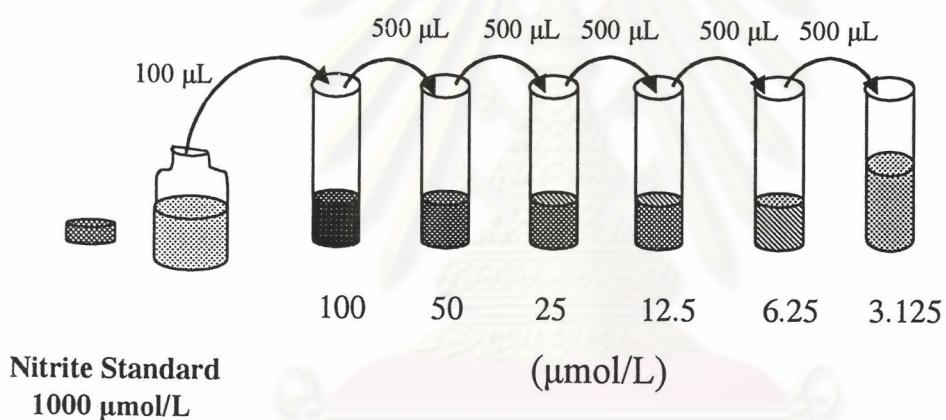
4. Nitrite Standard

Pipette 900 μL of Reaction Buffer (1x) into the 200 $\mu\text{mol/L}$ tube. Pipette 500 μL of Reaction Buffer (1x) into the remaining tubes. Use the 2000 $\mu\text{mol/L}$ standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 200 $\mu\text{mol/L}$ standard serves as the high standard and the Reaction Buffer (1x) serves as the zero standard (0 $\mu\text{mol/L}$).



5. Nitrate Standard

Pipette 900 μL of Reaction Buffer (1x) into the 100 $\mu\text{mol/L}$ tube. Pipette 500 μL of Reaction Buffer (1x) into the remaining tubes. Use the 1000 $\mu\text{mol/L}$ standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 100 $\mu\text{mol/L}$ standard serves as the high standard and the Reaction Buffer (1x) serves as the zero standard (0 $\mu\text{mol/L}$).



Describing Data (The mode)

The *mode* is the value that occurs most frequently. Even non-numerical data, for example, the immunohistochemistry staining scores or pathological scores. These scores can have a mode. There, considering the full set of 8 samples, the most frequency presence was 2, so that is the mode. In the present study, immunohistochemistry staining scores and pathological scores in each animal from each group were shown in Table 4 and Table 5, respectively

Table 4

The intensity scores of renal eNOS protein expression in cortex and medulla from left (obstruction) and right (non obstruction) kidney of rats in sham, UUO, UUO+ACEI, and UUO+ARA after 1 day or 7 days post UUO. Sections were scored in blinded, semiquantitative manner by three pathologists.

Groups	Cortex			Medulla			Cortex			Medulla			7 days			
	Pathologist			Pathologist			Pathologist			Pathologist			Pathologist			
	(1)	(2)	(3)	Mode	(1)	(2)	(3)	Mode	(1)	(2)	(3)	Mode	(1)	(2)	(3)	Mode
Sham	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
	2	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1
	3	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1
	4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
UUO	1	1-2	2	2	2	2	2	2-3	2	2	2	2	2	2	2	2
	2	2	2	2	1-2	2	1-2	2	2	2	2	2	2	2	2	2
	3	2	1-2	1-2	2	2	2	1-2	2	2	2	2	2	2	2	2
	4	1	1-2	1	1	2	1-2	1-2	2	2	2	2	1-2	1-2	1-2	1-2
+ water	5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
	6	2	1-2	1-2	2	2	2	2	2	2	2	2	1-2	1-2	1-2	1-2
	7	1	1-2	1-2	2	1-2	1	1-2	1-2	1-2	1-2	1-2	1	2	2	2
	8	2	2	2	2	2	2	2	2	2	2	2	1-2	2	2	2
UUO	1	1-2	1-2	1-2	1-2	2	1-2	1-2	2	2	2	2	3	3	2	3
	2	1-2	2	1-2	1-2	2	2	2	2	1-2	1-2	2	2-3	2	2-3	2-3
	3	2	2	2	2	1-2	1-2	1-2	2	2	2	2	2-3	2	2-3	2-3
	4	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	3	3	3	3
+ ACEI	5	1-2	1-2	1-2	1-2	2	2-3	2-3	2-3	2	2	2	3	3	3	3
	6	2	1-2	2	2	1-2	2	2	2	2	2	2	2-3	3	3	3
	7	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2	2-3	2-3	2-3
	8	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2	2	2	3	3	3	3
UUO	1	1-2	1-2	1-2	1-2	2	2	1-2	2	2	2	2	3	3	3	3
	2	1-2	1-2	1-2	1-2	2	1-2	1-2	2	2	2	2	3	3	3	3
	3	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2-3	3	2-3	3	3	3	3
	4	2	2	1-2	1-2	2	2	2-3	2	2	2	2	2	3	3	3
+ ARA	5	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2-3	2-3	2-3	2-3
	6	2	1-2	2	2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2	2	2	2
	7	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	1-2	2-3	3	2-3	2-3
	8	1-2	2	2	2	2-3	2-3	2	2	2	2	2	3	3	3	3

Table 5

The pathological scores of renal cortex and medulla from left (obstruction) and right (non obstruction) kidney of rats in sham, UUO, UUO+ACEI, and UUO+ARA after 1 day or 7 days post UUO. Sections were scored in blinded, semiquantitative manner by three pathologists.

Groups	Cortex			Medulla			Cortex			Medulla		
	Pathologist			Pathologist			Pathologist			Pathologist		
	(1)	(2)	(3)	Motif	(1)	(2)	(3)	Motif	(1)	(2)	(3)	Motif
Sham	1	0	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	2	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	3	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1	0-1
	4	0-1	0-1	0	0-1	0	0-1	0-1	0	0-1	0-1	0-1
UUO + water	1	2	2-3	2-3	2	2	2	3	2	2	2	2
	2	2-3	2-3	3	2-3	2-3	2-3	2-3	3	3	3	3
	3	2-3	3	3	2	2-3	2	2-3	3	3	3	3
	4	2-3	3	2-3	2-3	2-3	2-3	2-3	3	2-3	2-3	3
UUO + ACEI	5	2	2-3	2-3	2-3	2-3	2-3	2-3	3	2-3	2-3	3
	6	3	2-3	2-3	2-3	2-3	2-3	2-3	3	2-3	2-3	3
	7	2-3	2-3	2-3	2-3	2-3	2-3	2-3	3	2-3	2-3	3
	8	2-3	2	2-3	2-3	3	3	3	2-3	2-3	2-3	3
UUO + ARA	1	1-2	1-2	1-2	1	1-2	1	1-2	1-2	1	1-2	1
	2	1-2	2	2	1-2	1-2	1-2	1-2	1	1-2	1-2	1-2
	3	1-2	1-2	2	1-2	1-2	1-2	1-2	2	1-2	2	1-2
	4	1	1	1-2	1	1-2	1	1-2	2	1-2	1-2	1-2
UUO + ARA	5	2	1-2	1-2	1-2	2	2	2	1	1-2	1	2
	6	1	2	2	1-2	1-2	1	1-2	2	1-2	2	2
	7	2	1-2	1-2	2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
	8	1-2	1-2	1-2	2	1-2	1-2	1-2	1-2	1-2	1-2	1-2
UUO + ARA	1	2	1-2	2	1-2	2	2	2-3	2-3	2-3	3	3
	2	1	1	2	1	1-2	1-2	1-2	2-3	2-3	2-3	2-3
	3	1-2	1-2	1-2	2	2	1	2	2-3	2-3	2-3	2-3
	4	1-2	2	1-2	1-2	2	1-2	1-2	2-3	2-3	2-3	2-3
UUO + ARA	5	2	1-2	1-2	1-2	1	1-2	1-2	2-3	2-3	2-3	2-3
	6	1-2	2	2	2	1-2	1-2	1-2	2	2	2-3	2-3
	7	1	1-2	1-2	1-2	2	1-2	1-2	2-3	3	3	3
	8	1-2	2	1-2	1-2	1	1-2	1-2	2-3	2-3	2-3	2-3

BIOGRAPHY

Miss. Jintana Tanyong was born on November 14, 1977 in Singburi province, Thailand. She received the Bachelor degree of Science in Physical Therapy in 1999 from Srinakharinwirot University, Bangkok, Thailand. She has enrolled at Chulalongkorn University in graduate program for the Degree of Master of Science in Physiology and graduated in 2004.

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