APPENDIX

Preparation of reagent used in the study

1. 4M Guanidine hydrochloride (4M GuHCl) extraction and inhibitors.

GuHCl	191.05 g
Tris (base)	3.03 g
EDTA	4.655 g
e-aminocaporic acid	1.64 g
N-ethymeleimide	0.625 g
Benzamidine-HCl	0.92 g

All reagents were dissolved in distilled water and adjust pH to 7.4 with 1M HCl and made up volume to 500 ml.

2. Phosphate buffer saline (PBS)

NaCl	8.00 g
KCl	0.20 g
Na_2HPO_4	1.44 g
Na_2PO_4	0.24 g

All reagents were dissolved in distilled water and made up volume to 1 L. Adjusted the pH to 7.4 with HCl or NaOH and then added distilled water to adjust the volume to 1.000 ml and stored at room temperature. For PBS - Tween, Tween-20 was added 0.05%.

3. Citrate phosphate buffer

Citric acid monohydrate	10.30 g
$Na_2HPO_4 \cdot 3H_2O$	18.16 g

All reagents were dissolved in 900 ml of distilled water, adjusted pH to 5.0 and made up volume to 1 L. Stored reagent at 4°C.

4. 1.5 M Tris-HCl pH 8.8

Tris-base	18.15 g

reagent were dissolved in 95 ml of distilled water, adjusted pH to 8.8 and made up volume to 100 ml. Stored reagent at 4°C.

5. 0.5 M Tris-HCl, pH 6.8

Tris-base	6.0 g

Reagent was dissolved in 95 ml of distilled water. adjusted pH to 6.8 and made up volume to 100 ml. Stored reagent at 4°C.

6. Running Buffer, pH 8.3 (10x)

Tris-base	30.3 g
Glycine	144.0 g

All reagents were dissolved in 1,000 ml with deionized water. Do not adjust pH.

7. Sample Bufffer

0.5 M Tris-HCl, pH 6.8	1.0 ml
Glycerol	2.0 ml
0.5% Bromophenol blue	0.2 ml

All reagents were dissolved in 4.8 ml of distilled water.

8. Substrate solution

OPD	8 mg
Citrate phosphate buffer	12 ml
30% H_2O_2	5 µl

Prepare reagent fresh for 1 plate; keep in dark before use.

9. Acrylamide/Bis (30% T, 2.67%C)

Acylamide	29.2 g
N'N'-bis-methylene-acrlamide	0.8 g

All reagents were dissolved in 100 deionized water. store at 4 ^{0}C in the dark.

10. T-Gel binding buffer

Potassium sulfate	50 mM
Sodium phosphate	50 mM

All reagents were dissolved in 1,000 ml of distilled water.

11. T-Gel Elution Buffer

Sodium phosphate	50 mM

Reagent was dissolved in 1,000 ml of distilled water.

12. T-Gel cleaning buffer

Guanidine hydrochloride	230 g

Reagent was dissolve in 300 ml of distilled water.

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