APPENDIX A

REAGENTS AND SOLUTIONS

1. Reagents for routine plating and culture of *E. coli*

1.1 Kanamycin stock solution (500×)

The solution was prepared by dissolving 25 mg of kanamycin in one milliliter of DDW, mixed by inverting until solution becomes clear and then stored at -20°C.

1.2 Tetracycline stock solution (1000×)

The solution was prepared by dissolving 15 mg of tetracycline in one milliliter of DDW, mixed by inverting until solution becomes clear and then stored at -20°C

1.3 Chloramphenicol stock solution (100×)

The solution was prepared by dissolving 15 mg of chloramphenicol in one milliliter of absolute ethanol, mixed by inverting until solution becomes clear and then stored at -20°C

1.4 Ampicillin stock solution (2000×)

The solution was prepared by dissolving 100 mg of ampicillin in one milliliter of DDW, mixed by inverting until solution becomes clear and then stored at -20°C

1.5 LB broth

Bacto peptone	10	g
Bacto yeast extract	5	g
NaCl	5	g

The broth was prepared by dissolving all of the above reagents in DW to final volume of one liter, mixed until complete dissolving and then sterilized by autoclave.

1.6 LB agar plates

Bacto peptone	10	g
Bacto yeast extract	5	g
NaCl	5	g

Agar	15	g

The agar was prepared by dissolving all of the above reagents in DW to final volume of one liter, mixed until complete dissolving and then autoclaved. The solution was poured into bacterial culture plates, let them harden at room temperature and stored at 4°C.

1.7 LB/tet agar plates

To one liter of autoclaved LB agar, added one milliliter of tetracycline stock solution (15 mg/ml) after cooling to 50°C. The solution was carefully mixed by swirling and poured into bacterial culture plates. Let them harden at room temperature and stored at 4^{o}C.

1.8 LB/kan/cam agar plates

To one liter of autoclaved LB agar, added 2 ml of kanamycin stock solution (25 mg/ml) and 10 ml of chloramphenicol stock solution (15 mg/ml) after cooling to 50°C. The solution was carefully mixed by swirling and poured into bacterial culture plates. Let them harden at room temperature and stored at 4°C.

1.9 LB/amp agar plates

To one liter of autoclaved LB agar, added 0.5 ml of ampicillin stock solution (100 mg/ml) after cooling to 50°C. The solution was carefully mixed by swirling and poured into bacterial culture plates. Let them harden at room temperature and stored at 4°C.

2. Reagents for transduction and titering of λ phage in *E. coli*

2.1 $MgSO_4$ stock solution (1 M)

The stock solution was prepared by dissolving 24.65 g of $MgSO_4 \cdot 7H_2O$ in 100 ml of DDW and sterilized by filtering through a 0.22 μm membrane filter.

2.2 Tris-HCl, pH 7.5 stock solution (1 M)

The solution was prepared by dissolving 242.28 g of Tris base in a volume of distilled water. The pH was adjusted to 7.5 with HCl, brought up to final volume of one liter with DDW and then autoclaved.

2.3 Maltose stock solution (20% maltose)

The stock solution was prepared by dissolving 20 g of maltose in 80 ml DDW and mixed well. The volume was brought up to final volume of 100 ml with DDW, sterilized by filtering through a 0.22 μm membrane filter and stored at 4°C.

2.4 LB/$MgSO_4$ agar plates

To one liter of LB broth, 10 ml of $MgSO_4$ stock solution (1 M) and 15 g of agar were added. The solution was autoclaved, poured into bacterial culture plates and stored at 4°C.

2.5 LB/$MgSO_4$ broth

To one liter of LB broth, 10 ml of $MgSO_4$ stock solution (1 M) was added and then autoclaved.

2.6 LB/$MgSO_4$/maltose broth

One liter of LB/MgSO4 broth was prepared as described above (section 2.5). After autoclaving, the solution was cooled to 50°C before adding 10 ml of maltose stock solution.

2.7 LB/MgSO4 soft top agar

To one liter of LB broth, 10 ml of $MgSO_4$ stock solution (1 M) and 8 g of agarose were added. The solution was autoclaved and store at 4°C.

2.8 10× Lambda dilution buffer (1.0 M NaCl, 0.1 M $MgSO_4 \cdot 7H_2O$, 0.35 M Tris-HCl pH 7.5)

	Final concentration	To prepare 1 L of solution
NaCl	1.0 M	53.8 g
$MgSO_4 \cdot 7H_2O$	0.1 M	24.65 g
Tris-HCl (pH 7.5)	0.35 M	350.0 ml of 1 M

The buffer was prepared by dissolving all of the above reagents in DDW to a final volume of one liter. The buffer was autoclaved and stored at 4°C.

2.9 1× Lambda dilution buffer

The buffer was prepared by mixing 100 ml of 10x lambda dilution buffer and 5 ml of 2% gelatin. DDW was added to final volume of one liter, autoclaved and stored at 4°C.

3. Reagent for agarose gel electrophoresis

3.1 Ethidium bromide stock solution (10 mg/ml)

One gram of ethidium bromide was dissolved in 100 ml of DDW, mixed until solution becomes homogeneous and stored at 4°C.

3.2 TBE stock buffer (5×)

Tris-base	52.00	g
Boric acid	27.50	g
Disodium EDTA·$2H_2O$	4.65	g

All components were dissolved with DDW to final volume of one liter and mixed until solution becomes clear.

3.3 TBE running buffer (0.5×)

The TBE stock solution (Section 3.2) 100 ml and ethidium bromide stock solution (section 3.1) 50 µl were diluted with DDW to final volume of one liter and mixed until homogeneous.

3.4 Native agarose gel (0.7-1.2%)

To prepare agarose gel, 0.7-1.2 g of agarose powder was mixed with 100 ml of TBE running buffer (section 3.3) and heated by a microwave oven until agarose was completely melted and then added DDW to bring volume back to 100 ml again. The mixture was poured into the gel cassette after solution was cooled to 50°C. Allowing gel to harden for at least 45 minutes before use.

3.5 Sample loading buffer (10×)

Bromophenol blue	0.025	g
Xylene cyanon FF	0.025	g
Glycerol	5	ml
DDW	5	ml

All components were mixed by inverting until solution become homogeneous and then stored at 4°C. One microliter of sample loading buffer (10×) was mixed with nine microliter of DNA sample prior loading into agarose gel.

4. Reagents for isolation of plasmid DNA from *E. coli*

4.1 Tris-HCl, pH 8.0 stock solution (2 M)

The solution was prepared by dissolving 242.28 g of Tris base in a volume of DDW. The pH was adjusted to 8.0 with HCl, added DDW to final volume of one liter and autoclaved.

4.2 Glucose stock solution (2 M)

The solution was prepared by dissolving 36 g of glucose in 100 ml of DDW. The solution was sterilized by filter and stored at -20°C.

4.3 EDTA stock solution (0.5 M)

The solution was prepared by dissolving 18.6 g of EDTA·Na_2·$2H_2O$ in a volume of DDW. The pH was adjusted to 8.0 with NaOH, added DDW to final volume of 100 ml and autoclaved.

4.4 Solution I (25mM Tris pH 8.0, 50 mM glucose, 10 mM EDTA)

2.0 M Tris-HCl, pH 8.0	1.25	ml
2.0 M Glucose	2.50	ml
0.5 M EDTA pH 8.0	200	µl

All components were diluted with DDW to final volume of 100 ml and mixed until solution becomes homogeneous.

4.5 Solution II (0.1 N NaOH, 1% SDS) (freshly prepare)

The solution was freshly prepared by mixing one milliliter of 1 N NaOH with 0.5 ml of 20% SDS and then DDW was added to bring the final volume to 10 ml.

4.6 Solution III (2.7 M Potassium acetate, pH 4.8)

The solution was prepared by dissolving 26.5 g of potassium acetate in a volume of DDW. The solution was adjusted the pH to 4.8 and added DDW to bring the final volume to 100 ml.

4.7 70% ethanol

70% ethanol was prepared by dissolving 70 ml of absolute ethanol with 30 ml of DDW.

4.8 TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA)

The buffer was prepared by diluting 0.5 ml of 2.0 M Tris-HCl pH 8.0 and 0.2 ml of 0.5 M EDTA in DDW to final volume of 100 ml and then sterilized by autoclave.

5. Reagents for preparation of competent cell and transformation

5.1 $MgCl_2$ stock solution (1 M)

The solution was prepared by dissolving 9.5 g of $MgCl_2$ (anhydrous) in DDW to final volume of 100 ml and sterilized by filtering through a 0.22 µm membrane filter.

5.2 $CaCl_2$ stock solution (1 M)

The solution was prepared by dissolving 11.0 g of $CaCl_2$ (anhydrous) in DDW to final volume of 100 ml and sterilized by filtering through a 0.22 μm membrane filter.

5.3 $MgCl_2$ working solution (0.1 M)

The solution was prepared by diluting 10 ml of 1 M $MgCl_2$ stock solution in sterile DDW to final volume of 100 ml.

5.4 $CaCl_2$ working solution (0.1 M)

The solution was prepared by diluting 10 ml of 1 M $CaCl_2$ stock solution in sterile DDW to final volume of 100 ml.

5.5 SOB medium

Bacto peptone	20	g
Bacto yeast extracts	5	g
NaCl	0.5	g

All above were dissolved in DDW to final volume of 980 ml and mixed to completely dissolve. The medium was sterilized by autoclave and then added 10 ml of 1 M $MgSO_4$ (10 mM final concentration).

5.6 SOC medium

The medium was prepared by mixing 999 ml of SOB medium and 1 ml 2M glucose stock solution. The medium was aliquoted in 15 ml conical tube and stored at -20°C.

6. Reagents for DNA isolation from DEAE membrane

6.1 EDTA equilibrating solution (10 mM)

The solution was prepared by diluting two milliliter of 0.5 M EDTA, pH 8.0 in DDW to final volume 100 ml.

6.2 NaOH stock solution (5 N)

The solution was prepared by dissolving 20 g of NaOH tablet with DDW to final volume of 100 ml.

6.3 0.5 N NaOH solution (0.5 N)

The solution was prepared by diluting 10 ml of 5 M NaOH in DDW to final volume of 100 ml.

6.4 NaCl stock solution (5 M)

The solution was prepared by dissolving 29.2 g of NaCl in DDW to final volume of 100 ml and sterilized by autoclave.

6.5 Low-salt buffer (50 mM Tris-HCl pH 8.0, 0.15 M NaCl, 10 mM EDTA)

1 M Tris-HCl, pH 8.0	5	ml
5 M NaCl	3	ml
0.5 M EDTA	2	ml

All stock solutions were diluted with DDW to final volume of 100 ml and sterilized by autoclave.

6.6 High-salt buffer (50 mM Tris-HCl pH 8.0, 1 M NaCl, 10 mM EDTA)

1 M Tris-HCl, pH 8.0	5	ml
5 M NaCl	20	ml
0.5 M EDTA	2	ml

All stock solutions were diluted with DDW to final volume of 100 ml and sterilized by autoclave.

6.7 Ammonium acetate stock solution (10 M)

The solution was prepared by dissolving 77 g of ammonium acetate with DDW to final volume of 100 ml and sterilized by autoclave.

6.8 95% ethanol

95% ethanol was prepared by dissolving 95 ml of absolute ethanol with 5 ml of DDW.

7. Reagents for immobilization of bacteriophage onto nitrocellulose membrane, Southern hybridization and Northern hybridization

7.1 Denaturing solution (0.5 N NaOH, 1.5 M NaCl)

The solution was prepared by diluting 100 ml of 5 M NaOH and 300 ml of 5 M NaCl in DW to final volume of one liter.

7.2 Neutralizing solution (0.5 M Tris-HCl, pH 8.0, 1.5 M NaCl)

The solution was prepared by diluting 250 ml of 2 M Tris-HCl, pH 8.0 and 300 ml of 5 M NaCl in DW to final volume of one liter.

7.3 Sodium citrate stock solution (1 M, pH 7.0)

The solution was prepared by dissolving 294 g of sodium citrate in a volume of DW. Then pH was adjusted to desired pH (pH 7.0) with 1 M citric acid and added DW to final volume of one liter.

7.4 20× SSC stock solution (3 M NaCl, 0.3 M Sodium Citrate pH 7.0)

The solution was prepared by added 175.3 g of NaCl and 300 ml of 1 M sodium citrate, pH 7.0 and then added DW to final volume of one liter.

7.5 2× SSC (0.3 M NaCl, 30 mM Sodium Citrate pH 7.0)

The solution was prepared by diluting 100 ml of 20× SSC in DW to final volume of one liter.

7.6 5× SSC (0.75 M NaCl, 75 mM Sodium Citrate pH 7.0)

The solution was prepared by diluting 250 ml of 20× SSC in DW to final volume of one liter.

7.7 Denhardt's solution (100×)

Ficoll	2	g
Polyvinylpyrrolidon	2	g
BSA (fraction V)	2	g

All components were dissolved in DW to final volume of 100 ml and sterilized by filtering through membrane filter. The solution was aliqoted to 50 ml conical tubes and stored at -20°C.

7.8 Sodium phosphate buffer ($NaPO_4$; 0.5 M)

Solution A: 69 g $NaH_2PO_4 \cdot H_2O$ per liter (0.5 M)

Solution B: 89 g $Na_2HPO_4 \cdot 2H_2O$ per liter (0.5 M)

The buffer was prepared by titration of solution A and B to desired pH.

7.9 Hybridization solution (50% formamide, 5×SSC, 20mM phosphate buffer, 5× Denhardt's solution, 100 μg/ml heat-denatured Hering sperm DNA, 0.5% SDS)

100% Formamide	50	ml
20× SSC	25	ml
0.5 M phosphate buffer	4	ml
100× Denhardt's solution	5	ml
100 μg/ml Hering sperm DNA (heat-denatured 5 min at 100°C)	0.5	ml
20% SDS	2.5	ml

All solutions were mixed and then DW was added to bring up the final volume of 100 ml.

7.10 Washing buffer 1 (2× SSC, 0.1% SDS)

The buffer was prepared by diluting 100 ml of 20× SSC and 0.5 ml of 20% SDS with DW to final volume of one liter.

7.11 Washing buffer 2 (0.1× SSC, 0.1% SDS)

The buffer was prepared by diluting 0.5 ml of 20× SSC and 0.5 ml of 20% SDS with DW to final volume of one liter.

7.12 Maleic acid buffer (0.1 M Maleic acid, 0.15 M NaCl, pH 7.5)

The buffer was prepared by adding 11.6 g of maleic acid and 30 ml of 5 M NaCl in a volume of DW, mixed until dissolve and the pH is adjusted to 7.5 with NaOH tablet. The final volume was brought up to one liter with DW.

7.13 Maleic washing buffer (0.1 M Maleic acid, 0.15 M NaCl, pH 7.5, 0.3% Tween 20)

The buffer was prepared by adding 0.3 ml of Tween 20 to 100 ml of maleic acid buffer and mixed well.

7.14 Blocking reagent (10×)

10 g of blocking powder (Roche, Penzberg, Germany) was dissolved in maleic acid buffer to final volume of 100 ml with shaking and heating either on heating block or in a microwave oven and then autoclaved the stock solution.

7.15 Blocking solution (1×)

1× working solution was prepared by diluting 10 ml of 10× blocking reagent in maleic acid buffer to final volume of 100 ml.

7.16 Detection buffer (0.1 M Tris-HCl, 0.1 M NaCl, pH 9.5)

The buffer was prepared by dissolving 12.1 g of Tris base and 5.8 g of NaCl with a volume of DW, mixed and adjusted pH to 9.5 with HCl. The final volume was brought up to one liter with distilled water.

7.17 3-Morpholinopropane-sulfonic acid stock solution (1 M MOPS, pH 7.0)

The solution was prepared by dissolving 209.26 g of 3-Morpholinopropane-sulfonic acid with a volume of DW, mixed and adjusted pH to 7.0 with NaOH. The final volume was brought up to one liter with distilled water.

7.18 Sodium Acetate stock solution (3 M)

The solution was prepared by dissolving 24.6 g of sodium acetate with DW to final volume of 100 ml.

7.19 10× MOPS buffer (0.2 M 3-Morpholinopropane-sulfonic acid pH 7.0, 50 mM Sodium Acetate, 10 mM EDTA)

1 M MOPS	200	ml
3 M Sodium acetate	16.67	ml
0.5 M EDTA	20	ml

All stock solutions were diluted with DW to final volume of one liter.

7.20 1× MOPS (Running buffer)

The buffer was prepared by diluting 100 ml of 10× MOPS with DW to final volume of one liter.

7.21 DEPC-treated water (0.1%)

The RNase free water was prepared by adding one milliliter of diethyl pyrocyanate (DEPC) into one liter of DDW, mixed well by shaking and incubated overnight in fume hood. The DEPC was eliminated by autoclave.

7.22 RNA sample loading buffer (2×)

The sample buffer was prepared by mixing 30 mg of bromophenol blue, 5 ml of glycerol and 5 ml of DEPC-treated water.

7.23 Formaldehyde-denaturing agarose gel (2.2 M Formaldehyde, 1% Agarose gel)

One gram of agarose gel was dissolved in 71 ml DEPC-treated water by heating with a microwave oven and then added 10 ml of 10× MOPS and 18 ml of 38% Formaldehyde (12.6 M) after temperature cool to 50°C. The mixture was mixed well and immediately poured into the gel cassette that was prepared in the fume hood. Allowing gel to harden for at least 45 minutes before use.

8. Reagents for parasite preparation and nucleic acid extraction

8.1 Normal saline solution (NSS; 0.85% NaCl)

The solution was prepared by dissolving 8.5 g of NaCl in DW to final volume of one liter.

8.2 Homogenization buffer (30 mM Tris-HCl pH 8.0, 0.1 M NaCl, 10 mM EDTA, 0.5% Triton X-100)

2 M Tris-HCl, pH 8.0	1.5	ml
5 M NaCl	2	ml
0.5 M EDTA	2	ml

All solutions were diluted in DDW to final volume of 100 ml and then added 0.5 ml of Triton X-100. The solution is mixed by shaking until clear and homogeneous.

8.3 Extraction buffer (0.1 M Tris-HCl pH 8.0, 0.1 M NaCl, 20 mM EDTA)

2 M Tris-HCl, pH 8.0	5	ml
5 M NaCl	2	ml
0.5 M EDTA	4	ml

All solutions were diluted in DDW to final volume of 100 ml.

8.4 75% Ethanol-DEPC

75% ethanol was prepared by diluting 75 ml of absolute ethanol with 25 ml of DEPC-treated water.

9. Reagents for Tissue preparation and *in situ* hybridization

9.1 Phosphate buffer saline stock solution (1.37 M NaCl, 27 mM KCl, 100 mM Na_2HPO_4, 20 mM KH_2PO_4, pH 7.4; 10× PBS)

NaCl	80	g
KCl	2	g
Na_2HPO_4	14.4	g
KH_2PO_4	2.4	g

All above were dissolved with a volume of DDW and pH was adjusted to 7.4 with HCl. The final volume was brought up to one liter with DDW and then sterilized by autoclave.

9.2 Phosphate buffer saline (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4; 1× PBS)

The buffer was prepared by diluting 100 ml of 10× PBS with sterile DDW to final volume of one liter.

9.3 Fixative solution (4% Paraformaldehyde [PFA])

The DW was heated to 60°C a volume of water equal to slightly less than 2/3 the desired final volume of fixative. The paraformaldehyde was weighted 4 g and added it with stir bar to the DW. The container was covered and transferred to fume hood and maintained on heating plate at 60°C with stirring. The solution was clarified by adding one drop of 2 N NaOH, removed from heat and added 10 ml of 10× PBS. The pH was brought to 7.2 with HCl, added DW to final volume of 100 ml, filtered and cooled to room temperature.

9.4 25% Ethanol-DEPC

25% ethanol was prepared by dissolving 25 ml of absolute ethanol with 75 ml of DEPC-treated water.

9.5 50% Ethanol-DEPC

50% ethanol was prepared by dissolving 50 ml of absolute ethanol with 50 ml of DEPC-treated water.

9.6 70% Ethanol-DEPC

70% ethanol was prepared by dissolving 70 ml of absolute ethanol with 30 ml of DEPC-treated water.

9.7 80% Ethanol-DEPC

80% ethanol was prepared by dissolving 80 ml of absolute ethanol with 20 ml of DEPC-treated water.

9.8 95% Ethanol-DEPC

95% ethanol was prepared by dissolving 95 ml of absolute ethanol with 5 ml of DEPC-treated water.

9.9 LiCl stock solution (4 M)

The solution was prepared by dissolving 16.9 g of LiCl in DDW to final volume of 100 ml and sterilized by filtering through a 0.22 μm membrane filter.

9.10 1× DEPC-treated PBS

The RNase free PBS was prepared by adding 1 ml of diethyl pyrocyanate (DEPC) into one liter of 1× PBS, mixed well by shaking and incubated overnight in fume hood. The DEPC was eliminated by autoclaved

9.11 Washing buffer (0.1% Tween-20 in PBS, pH 7.4; PBST)

The buffer was prepared by adding one milliliter of Tween 20 into one liter of 1x DEPC-treated PBS and mixed well by shaking.

9.12 1× PBST, 10 mg/ml glycine

The buffer was prepared by dissolving 100 mg of glycine with one liter of 1× PBST and mixed well by shaking.

9.13 4× SSC, 0.1%SDS washing solution

The washing solution was prepared by diluting 200 ml of 20× SSC and 5 ml of 20% SDS with DEPC-treated water to final volume of one liter.

9.14 1× SSC, 0.1%SDS

The washing solution was prepared by diluting 50 ml of 20× SSC and 5 ml of 20% SDS with DEPC-treated water to final volume of one liter.

9.15 0.5× SSC, 0.1%SDS

The washing solution was prepared by diluting 25 ml of 20× SSC and 5 ml of 20% SDS with DEPC-treated water to final volume of one liter.

9.16 NTE buffer (0.5 M NaCl, 10 mM Tris, 1 mM EDTA, pH 8.0)

5 M NaCl	10	ml
2 M Tris-HCl, pH 8.0	0.5	ml
0.5 M EDTA	0.2	ml

All solutions were diluted in DEPC-treated water to final volume of 100 ml

9.17 Buffer 1 (100 mM Tris-HCl,150 mM NaCl, pH 7.5)

The buffer 1 was prepared by diluting 100 ml of 1 M Tris-HCl, pH 7.5 and 30 ml of 5 M NaCl with DEPC-treated water to final volume of one liter.

9.18 Buffer 1 containing 0.05% Tween20

The buffer was prepared by adding 50 μl of Tween 20 into 100 ml of buffer 1 and mixed well by shaking.

9.19 Buffer 2 (100 mM Tris-HCl pH 9.5, 50 mM $MgCl_2$, 100 mM NaCl, 0.1% Tween 20)

1 M Tris-HCl, pH 9.5	10	ml
1 M MgCl	5	ml
5 M NaCl	2	ml

All solutions were diluted with DEPC-treated water to final volume of 100 ml, 100 μl of Tween 20 was added and mixed well by shaking.

10. Reagents for protein purification by Ni-NTA affinity chromotography under denaturing condition

10.1 Buffer B (100 mM NaH_2PO_4, 10 mM Tris-Cl, 8 M Urea, pH 8.0)

$NaH_2PO_4 \cdot H_2O$	13.8	g
Tris-base	1.2	g
Urea	480.5	g

All components were dissolved in a volume of DDW, adjusted pH with NaOH to 8.0 and then added DDW to final volume of one liter.

10.2 Buffer C (100 mM NaH_2PO_4, 10 mM Tris-Cl, 8 M Urea, pH 6.3)

$NaH_2PO_4 \cdot H_2O$	13.8	g
Tris base	1.2	g
Urea	480.5	g

All components were dissolved in a volume of DDW, adjusted pH with HCl to 6.3 and then added DDW to final volume of one liter.

10.3 Buffer D (100 mM NaH_2PO_4, 10 mM Tris-Cl, 8 M Urea, pH 5.9)

$NaH_2PO_4 \cdot H_2O$	13.8	g
Tris-base	1.2	g
Urea	480.5	g

All components were dissolved in a volume of DDW, adjusted pH with HCl to 5.9 and then added DDW to final volume of one liter.

10.4 Buffer C (100 mM NaH_2PO_4, 10 mM Tris-Cl, 8 M Urea, pH 4.5)

$NaH_2PO_4 \cdot H_2O$	13.8	g
Tris-base	1.2	g
Urea	480.5	g

All components were dissolved in a volume of DDW, adjusted pH with HCl to 4.5 and then added DDW to final volume of one liter.

11. Reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

11.1 Sample buffer (2× SDS reducing buffer)

The sample buffer was prepared as a stock solution by combination the following ingredients:

0.5 M Tris-HCl, pH 6.8	4.0	ml
Glycerol	0.8	ml
SDS (10% solution)	1.6	ml
0.05% Bromophenol blue	0.2	ml
2-6-mercaptoethanol	0.4	ml
DDW	4.0	ml

This mixture was stored at room temperature in small aliquots. One part of sample was diluted with at least 4 parts of the sample buffer and heated at 100°C for 4 min before loading into gel.

11.2 Stock acrylamide solution (30%)

To prepare this solution, 30 g of acrylamide and 0.8 g of N,N-methylacrylamide were dissolved in 100 ml of DDW. The solution was sterilized by filtering through a 0.22 μm membrane filter. This stock solution was stored at 4°C in a dark bottle.

11.3 Tris-HCl (1.5 M, pH 8.8)

To prepare this solution, 18.15 g of Tris base (hydroxymethyl) amino-methane was dissolved in 50 ml of DDW, then the pH was adjusted to 8.8 with 1 N HCl. The final volume was brought up to 100 ml with DDW. The solution was filtered through a 0.22 μm membrane filter. This stock solution was stored at 4°C.

11.4 Tris-HCl (0.5 M, pH 6.8)

To prepare this solution, 6.05 g of Tris base (hydroxymethyl) aminomethane was dissolved in 50 ml of DDW, then the pH was adjusted to 6.8 with 1 N HCl. The final volume was brought up to 100 ml with DDW. The solution was filtered through a 0.22 μm membrane filter. This stock solution was stored at 4°C.

11.5 Ammonium persulfate (10%; w/v)

This solution was prepared just before use by dissolving 50 mg of ammonium persulfate in 0.5 ml of DDW.

11.6 Water-saturated n-butanol

The water-saturated n-butanol was prepared by combination 50 ml of n-butanol and 5 ml of DDW in a bottle and shaken. Allow the phases to separate, used the top phase to overlayer gels and stored at room temperature indefinitely.

11.7 Separating gel (12%)

Polyacrylamide separating gel (12%) was prepared by mixing the following ingredients together:-

DDW	5.1	ml
1.5 M Tris-HCl, pH 8.8	3.75	ml
10% SDS solution	150	μl
30% stock acrylamide solution	6	ml

The reagents were gently mixed and degassed under a vacuum for 5 minutes. The polymerization was initiated by adding 75 μl of the 10% ammonium persulfate (freshly prepared) and 7.5 μl of TEMED. The gel was poured into the casting apparatus and was overlayed with water-saturated n-butanol.

11.8 Stacking gel (4%)

The stacking gel (4%) was prepared by mixing the following reagents:-

DDW	6.1	ml
0.5 M Tris-HCl, pH 6.8	2.5	ml
SDS (10% solution)	0.1	ml
and 30% stock acrylamide	1.3	ml

All reagents were mixed gently and degassed under a vacuum for 5 minutes, then 50 µl of freshly prepared 10% ammonium persulfate and 10 µl of TEMED were subsequently added. After complete mixing, the upper portion of the gel polymerized in the casting apparatus was rinsed with DDW, the comb was inserted between the glass plates over the polymerized separating gel and the mixture of stacking gel was poured on top of the separating gel. The stacking gel was allowed to polymerize at least 45 minutes at room temperature before use.

11.9 5× Electrode (running) buffer pH 8.3

The buffer contained the following reagents: 15 g of Tris base (hydroxy-methyl) aminomethane (Sigma Chemical Co., U.S.A.); 72 g of glycine and 5 g of SDS. The buffer was prepared by dissolving all of the above reagents in a volume of DDW then the volume was made up to one liter with DDW. The buffer was stored at 4°C.

11.10 Electrode (running) buffer (1×)

The 5× electrode buffer 60 ml was diluted with 240 ml of DDW. Each preparation of the running buffer was used for only one electrophoretic run.

12. Reagents for Staining of the gel using colloidal Coomassie blue G-250 solution.

12.1 Fixing solution (1.3% o-phosphoric acid, 20% methanol)

The solution was prepared by mixing 5 ml of 80% o-phosphoric acid, 100 ml of Methanol and 395 ml of DDW. Gel was fixed in fixing solution for one hour prior staining.

12.2 Staining stock solution A (2% o-phosphoric acid, 10% ammonium sulfate)

The solution was prepared by mixing 9.5 ml of 80% o-phosphoric acid and 40 g of ammonium sulfate. The final volume was made up to 400 ml with DDW.

12.3 Staining stock solution B (5% Coomassie Brilliant Blue G-250)

The solution was prepared by mixing 2.5 g of Coomassie Brilliant Blue G-250 with DDW to final volume of 50 ml.

12.4 Staining working solution

The solution was prepared by mixing 10 ml of staining stock solution B with 400 ml of staining stock solution A and then added 100 ml of Methanol. The staining solution was applied to gel after removing the fixing solution and shaken on orbital shaker overnight.

12.5 Neutralization solution (pH 6.5)

The solution was prepared by dissolving 6 g of Tris-base in a volume of DDW and then pH was adjusted with o-phosphoric acid to 6.5. The final volume was brought up to 500 ml with DDW. After staining process, all staining solution was removed and placed with neutralization solution for one hour.

12.6 Washing solution (25% methanol)

The solution was prepared by diluting 125 ml of methanol with DDW to final volume of 500 ml. Gel was washed briefly until bands of protein are cleared and no background.

12.7 Stabilizing solution (20% ammonium sulfate)

The solution was prepared by dissolving 100 g of ammonium sulfate in DDW to final volume of 500 ml. After removing the washing solution, the stabilizing solution was equilibrated to stabilize color.

13. Reagents for western blot analysis

13.1 Transfer buffer (blotting buffer, pH 8.3)

(25 mM Tris, 192 mM glycine and 20% [v/v] methanol)

To prepared 4,000 ml of this buffer, 12.12 g of Tris-base and 57.60 g of glycine were dissolved in 3,200 ml of DDW. Subsequently, 800 ml of methanol was added to yield 20% (v/v).

13.2 Phosphate buffered saline (0.01 M PBS, pH 7.4)

This solution was prepared by dissolving 1.216 g of anhydrous Na_2HPO_4, 0.17 g of anhydrous NaH_2PO_4 and 8.766 g of NaCl in one liter of DW. The pH of this solution was adjusted to 7.4 with 1 N HCl.

13.3 Substrate buffer (0.15 M Tris-HCl, pH9.5)

The buffer was prepared by diluting 150 ml of 1 M Tris-HCl, pH 9.5 with DDW to final volume of one liter.

13.4 Washing buffer (0.05% Tween-20 in PBS, pH 7.4; PBST)

This solution was prepared by adding 0.5 ml of Tween-20 in one liter of PBS, pH 7.4 and mixed well.

13.5 Blocking solution (3% BSA, 0.2% gelatin, in PBS, pH 7.4)

The solution was prepared by dissolving 0.2 g of gelatin (Sigma Chemical Co., USA) in 100 ml of PBS, pH 7.4 by heating on a hot plate. The solution was cooled down to room temperature and then added 3 g of bovine serum albumin (BSA, Sigma Chemical Co., USA). The solution was mixed until dissolve and stored at -20°C.

13.6 Diluent solution (0.2% BSA, 0.2% gelatin in PBS, pH 7.4)

The solution was prepared by dissolving 0.2 g of gelatin (Sigma Chemical Co., USA) in 100 ml of PBS, pH 7.4 by heating on a hot plate. The solution was cooled down to room temperature and then added 0.2 g of bovine serum albumin (BSA, Sigma Chemical Co., USA). The solution was mixed until dissolve and stored at -20°C.

13.7 Ponceau S staining solution

The staining solution was prepared by dissolving 0.25 g of Ponceau S in 100 ml of 1% (v/v) acetic acid in DW. The solution was mixed and stored at room temperature.

14. Reagents for Immunolocalization

14.1 Antigen retrieval solution (10 mM sodium citrate buffer pH 6.0)

The solution was prepared by diluting 10 ml of 1 M sodium citrate, pH 6.0 with DDW to final volume of one liter.

14.2 Blocking solution (10% normal goat serum, in PBS, pH 7.4)

The solution was prepared by mixing 10 ml of normal goat serum and 90 ml of 1× PBS, pH 7.4.

14.3 Diluent solution (1% normal goat serum, in PBS, pH 7.4)

The solution was prepared by mixing 1 ml of normal goat serum and 99 ml of 1x PBS, pH 7.4.

14.4 0.05 M TBS, pH 7.6

The solution was prepared by diluting 50 ml of 1 M TBS, pH 7.6 with DDW to final volume of one liter.

14.5 Avidin-Biotin Complex conjugated with HRP (ABComplex/HRP) (DakoCytomation, Denmark)

The complex solution was prepared by placing 5 ml of 0.05 M TBS, pH 7.6 in the ABComplex mixing bottle. Then reagent A containing avidin was added one drop following one drop of reagent B containing biotinyled peroxidase. The bottle was caped on and mixed well. Leave the solution for 30 minutes before use.

The ABComplex/HRP at working dilution is stable for 3 days at 4°C.

14.6 Aminoethyl carbazole substrate (AEP) (Zymed Laboratories Inc., Germany)

The substrate was prepared by adding one drop of reagent A to one milliliter of DDW, mixed well and then added one drop of reagent B and C and mixed again. The solution was kept away from light and used within 30 minutes.

15. Reagent for autocatalytic processing and activity assay

15.1 Sodium citrate stock solution (1 M)

The stock solution was prepared by mixing 1 M of sodium citrate solution with 1 M of citric acid solution to desired pH.

15.2 Assay buffer (50 mM sodium acetate, pH 5.4, 1 mM DTT)

The buffer was prepared by diluting 5 ml of 1 M sodium acetate and 1 ml of 100 mM DTT in sterile DDW to final volume of 100 ml.