



APPENDIX H

สำนักหอสมุด

Reagents for M13 phage biopanning and ScFv expression

1. Minimal medium agar (MM)

The agar consisted of the following ingredients:

Solution A

NaHPO ₄	6 g,
KH ₂ PO ₄	3 g,
NH ₄ Cl	1 g,
DW	500 ml

Solution B

Agar	15 g,
DW	500 ml

Both solutions were sterilized by autoclaving at 151 lb/inch², at 121°C for 15 min. The solutions were cooled to 50°C and combined. The following solutions were added; 1 ml of sterilized MgCl₂.6H₂O, 1 ml of 1 M CaCl₂.2H₂O, 1 ml of 1 M thiamine hydrochloride and 3 ml of 2 M glucose. The agar was poured immediately into petridishes. The agar plates were kept at 4°C.

2. 2× YT agar

The agar consisted of the following ingredients:

Tryptone	20 g,
Yeast extract	10 g,
NaCl	5 g,
Agar powder	10 g
Distilled water approximately	940 ml

The ingredients were dissolved by heating and sterilized by autoclaving at 151 lb/inch², at 121°C for 15 min. The medium was cooled to 50°C. The following solution were added; 10 ml of 1 M MgCl₂, 55.6 ml of 2 M glucose and ampicillin to final concentration 100 µg/ml. The agar was poured into petridishes. The agar plates were kept at 4°C.

3. 2× YT medium

The medium consisted of the following ingredients:

Tryptone	20 g,
Yeast extract	10 g,
NaCl	5 g,
Add distilled water to	1,000 ml

The ingredients were dissolved and sterilized by autoclaving at 151 lb/inch², at 121°C for 15 min. The medium was cooled to 50°C and kept at 4°C.

3. 2× YT-AG medium

The 2xYT medium contained 2% glucose and 100 µg/ml ampicillin.

4. 2× YT-AK medium

The 2xYT medium contained 100 µg/ml ampicillin and 50 µg/ml kanamycin.

5. 2× YT-ANG agar

The 2xYT agar contained 100 µg/ml ampicillin, 100 µg/ml nalidixic and 2% glucose.

6. 2× YT-AI medium

The 2xYT medium contained 100 µg/ml ampicillin and 1 mM IPTG.

7. TSS buffer

The solution consisted of the following ingredients:

Tryptone	1 g,
Yeast extract	0.5 g,
NaCl	0.5 g,
Polyethylene glycol (PEG, MW 3,350)	10 g
Dimethylsulfoxide (DMSO)	5 ml
1 M MgCl ₂	5 ml
Distilled water to	70 ml

The ingredients were dissolved by stirring. The pH was adjusted to 6.5 by adding HCl or NaOH. Distilled water was added to make 100 ml. the solution was sterilized by filtering through a 0.22 μm and kept at 4°C.

8. PEG/NaCl

The solution was prepared by dissolving 200 grams of polyethylene glycol 8000 and 146.1 g of NaCl in 1 liter of distilled water. The solution was sterilized by autoclaving at 151 lb/inch², at 121°C for 15 min and kept at 4°C.

9. TES buffer

The buffer consisted of the following ingredients:

Tris base	4.85 g
EDTA	37 mg
Sucrose	34.23 g
Add distilled water	150 ml

The ingredients were dissolved by stirring. The pH was adjusted to 8.0 by adding HCl. Distilled water was added to make 200 ml. The solution was sterilized by filtering through a 0.45 μm and kept at 4°C.