

Gel-shift Assay of Ternary Complex Formation (Byron Hetrick)

Adapted from (Bilgin, et. al. (1998) Biochemistry. v137. 8163-8172

Conditions of ternary complex formation can be changed as needed depending upon what is being tested. Standard EF-Tu activity assays involve incubation of constant (100pmol) of EF-Tu with varying (0-300pmol) of tRNA^{phe}. It typically takes roughly 3x excess to saturate binding as measured by this gel shift. Ternary complex formation conditions can best be tested by incubating fixed tRNA^{phe} (100pmol) with varying amounts of EF-Tu to determine the best ratio under the given conditions.

Running Buffer:

10mM MES	(1M)	10mL
10mM MgoAc	(1M)	10mL
65mM NH ₄ oAc	(3M)	21.6mL
1mM DTT	(1M)	1.0mL

- Raise volume to 800mL
- Adjust pH to 6.7 with 10M NaOH
- Final volume is 1L
- Store at 4°C until ready to run gel

Native Gel:

2X Native Gel Buffer:

20mM MES	(1M)	2mL
20mM MgoAc	(1M)	2mL
130mM NH ₄ oAc	(3M)	4.32mL

- Raise volume to ~80mL
- Adjust pH to 6.7 with 10M NaOH
- Final volume is 100mL

Native Gel Recipe:

2X Native Gel Buffer		5mL
1mM DTT	(1M)	10µL
5% Acrylamide	(40%)	1.25mL
Water		3.75mL
Final Volume		10mL

- Polymerize with 200uL 10% APS and 20uL TEMED
- Store at 4°C until ready to run

Ternary Complex Formation:

Conditions for a "standard" ternary complex formation reaction are listed below. Reaction conditions may change depending upon what is being tested

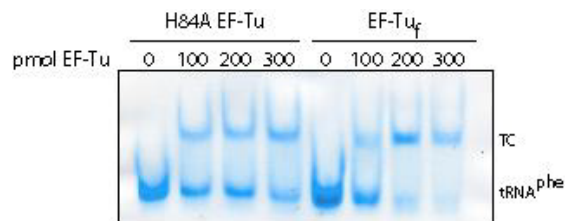
5X Reaction Buffer		4 µL
1mM Phenylalanine	(10mM)	2 µL
3mM ATP	(100mM)	0.6 µL
1mM GTP	(10mM)	2 µL

3mM Phosphoenol Pyruvate	(50mM)	1.2 μ L
Pyruvate Kinase		0.5 μ L
3% Phenylalanyl-tRNA Synthase		0.6 μ L
15uM EF-Tu	(varies)	varies
5uM tRNA ^{phe}	(varies)	varies
Final Volume		20 μ L

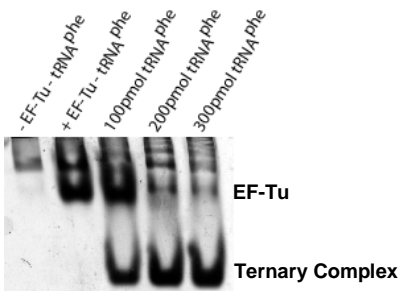
- Incubate 37°C for 1hour
- Add 0.1 vol. (2 μ L) 50% glycerol with trace bromphenol blue
- Load 20 μ L on native gel and run at 80mA, 200V, 4°C for 1hour
- Stain with methylene blue or coomassie blue

Examples of Gels:

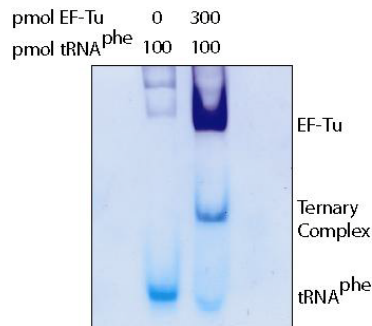
Gel stained with methylene blue:



Gel stained with coomassie blue:



Gel stained with coomassie and methylene blue:



I stained first with coomassie then with methylene blue after destaining