**Recipes for *in vitro* kinetics experiment**

**10x Transcription Buffer**

4 mL of 1 M Tris–HCl, pH 8.0 (final concentration = 400 mM)
4 mL of 1 M $MgCl_2$ (final concentration = 400 mM)
1 mL of 1 M DTT (final concentration = 100 mM)
400 µL of 500 mM Spermidine (final concentration = 20 mM)

Add water to 10 mL, filter to sterilize, and store at -20 °C.

**Crush soak buffer**

10 mL of 1 M Tris–HCl, pH 7.5 (final concentration = 10 mM)
40 mL of 5 M NaCl (final concentration = 200 mM)
2 mL of 0.5 M EDTA, pH 8.0 (final concentration = 1 mM)
dd$H_2O$ to 1 L

**10x Tris–Borate–EDTA (TBE) buffer**

108.99 g of Tris (final concentration = 900 mM)
55.64 g of Boric acid (final concentration = 900 mM)
20 mL of 0.5 M EDTA, pH 8.0 (final concentration = 10 mM)

Adjust pH to 8.3, and add dd$H_2O$ to 1 L.

**2x Urea gel loading buffer (ULB)**

2 g of sucrose (final concentration = 20%)
100 µL of 10% SDS (final concentration = 0.1%)
1 mL of 10x TBE (final concentration = 1x)
11 g of Urea (final concentration = ~18 M)

Add water to 10 mL, stir for 2 h at room temperature, decant the solution from any undissolved urea, filter to sterilize, and store at 4 °C.

**2x Urea gel loading buffer (ULB) with tracker dyes**

2 g of sucrose (final concentration = 20%)
5 mg of Bromophenol Blue (final concentration = 0.05%)
5 mg of Xylene cyanol (final concentration = 0.05%)
100 µL of 10% SDS (final concentration = 0.1%)
1 mL of 10x TBE (final concentration = 1x)

11 g of Urea (final concentration = ~18 M)

Add water to 10 mL, stir for 2 h at room temperature, decant the solution from any undissolved urea, filter to sterilize, and store at 4 °C.

**1x Tris–EDTA (TE) buffer**

100 µL of 1 M Tris–HCl, pH 7.5 (final concentration = 10 mM)
20 µL of 0.5 M EDTA, pH 8.0 (final concentration = 1 mM)

Add water to 10 mL, sterile filter, and store at room temperature.

**Inorganic pyrophosphatase solution**

100 µL of 50% Glycerol (final concentration = 25%)
10 µL of 1 M HEPES–KOH, pH 7.5 (final concentration = 50 mM)
0.5 µL of 200 mM DTT (final concentration = 0.5 mM)
100 U of Inorganic pyrophosphatase (final concentration= 0.5 U/µL)
89.5 µL of sterile dd$H_2O$

**10x Sodium carbonate buffer**

0.53 g of $Na_2CO_3$ (final concentration = 500 mM) 200
µL of 0.5 M EDTA (final concentration = 10 mM)

Adjust pH to 7.0, add water to 10 mL, sterile filter, and store at -20 °C.

**2x Renaturing buffer**

800 µL of 1 M HEPES–KOH, pH 7.5 (final concentration = 80 mM)
60 µL of 1 M MgCl2 (final concentration = 6 mM)
1.25 mL of 2M KCl (final concentration = 250 mM)

**DFHBI Stock**

1 mg of DFHBI (final concentration= 20 mM)198
µL of DMSO

Aliquot into amber tubes and store at -20 °C until used. Avoid repeated freeze-thaw cycles by usingone stock at a time and storing it at room temperature in the dark.

**<u>Recipes for *in vivo* kinetics experiment</u>**

**DFHBI-1T Stock**

5 mg of DFHBI-1T (final concentration = 50 mM)
312.3 µL of DMSO

Aliquot into amber tubes, and store at -20 °C until used. Avoid repeated freeze-thaw cycles by using one stock at a time and storing it at room temperature in the dark.

**Noninducing Media (NI), 200 mL media**

~ 186 mL of Sterile dd$H_2O$

After autoclaving, add the following sterile filtered components:

200 µL of 1 M $MgSO_4$
2.5 mL of 40% Glucose
10 mL of 20x NPS

**ZYP-5052 autoinduction media (AI), 200 mL media**

ZY Media
2 g of Tryptone
1 g of Yeast Extract
~ 186 mL of Sterile dd$H_2O$

After autoclaving, add the following sterile filtered components:

200 µL of 1 M $MgSO_4$
4 mL of 50x 5052
10 mL of 20x NPS

**1 M $MgSO_4$ solution, 50 mL**

6 g of $MgSO_4$
50 mL of dd$H_2O$

Add water to $MgSO_4$, and stir until fully dissolved. Sterile filter solution afterward before use.

**40% Glucose, 100 mL**

40 g of D-Glucose
100 mL of dd$H_2O$

Weigh out 40 g of glucose and add 70 mL of dd$H_2O$. Dissolve by stirring. May use some heat to assist with dissolving. Once the sugar has dissolved, bring the volume up to 100 mL total. Autoclave solution before use.

**20x NPS, 100 mL**

90 mL of Sterile ddH2O
6.6 g of $(NH_4)_2\ SO_4$ (final concentration = 0.5 M)
13.6 g of $KH_2PO_4$ (final concentration = 1 M)
14.2 g of $Na_2HPO_4$ (final concentration = 1 M)

Add in sequence into a breaker, stir until dissolved. pH of 20-fold dilution in water ~6.75. Autoclave solution before use.

**50x 5052, 100 mL**

25 g of Glycerol (weigh in beaker)
73 mL of Sterile $ddH_2O$
2.5 g of Glucose
10 g of α-lactose

Add in sequence in a beaker, and stir until dissolved. Can speed by heating in a microwave. Once dissolved, the solution is sterile filtered.