

July 14 2016

## F-Actin Preparation Unlabeled/Labeled

### Prepare Buffers

#### **G-Buffer (10mL)**

Type	[Stock]	[Final]	Vol to mix	Total vol
TRIS-HCl (pH 8.0)	1M	2mM	20 $\mu$ L	20 $\mu$ L
CaCl <sub>2</sub>	1M	0.2mM	2 $\mu$ L	22 $\mu$ L
ATP	106mM	0.2mM	21.6 $\mu$ L	43.6 $\mu$ L
DTT	1M	0.5mM	5 $\mu$ L	48.6 $\mu$ L
Filtered diH <sub>2</sub> O	X	X	9.95mL	10.0mL

#### **4X F-Buffer (10mL)**

Type	[Stock]	[Final]	Vol to mix	Total vol
KCl	3M	300mM	1mL	1mL
MgCl <sub>2</sub>	1M	10mM	100 $\mu$ L	1.1mL
HEPES (pH 7.2)	1M	40mM	400 $\mu$ L	1.5mL
Filtered diH <sub>2</sub> O	X	X	8.5mL	10.0mL

### F-Actin Polymerization

#### **1 $\mu$ M F-Actin (500 $\mu$ L)**

1. Add 2.08 $\mu$ L of 240 $\mu$ M G-actin (500pmol final concentration)
  - a. G-actin can be unlabeled or labeled; sparse Alexa actin is 20% labeled and 80% unlabeled
2. Add G-buffer to 20 $\mu$ L
3. Mix well; Invert gently, do not shake
4. Add 125 $\mu$ L of 4X F-Buffer
5. Add 42 $\mu$ L of 13.2 $\mu$ M phalloidin (550pmol)
  - a. Phalloidin can be unlabeled or labeled
6. Add diH<sub>2</sub>O to total volume of 500 $\mu$ L (313 $\mu$ L)
7. Invert gently to mix
8. Store at room temperature for 30 minutes

### Storage

1. Store at 4°C overnight prior to use
2. Stable at 4°C for up to 3 weeks