# Myosin Motility Assay

Thank Deb!

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> On Jul 31, 2015, at 1:41 PM, Deborah Shroder <deborah.shroder@gmail.com> wrote:
>
> Solutions to make:
> (all aqueous stock solutions should be filtered through 0.2 um
> filters)
>
> #PMMA ~2mg/mL in methylene chloride
>
> -> Dip 1mL pipette tip into PMMA powder until approx. 1 mm at the end of pipette tip. Transfer to clean tube.
> -> Rinse glass Pasteur pipette 3X with methylene chloride. Add ~1mL
> methylene chloride to PMMA tube
>
> #Myosin 5 buffer (M5B) + 10 mM DTT
>
> M5B: 25 mM KCl, 50 mM HEPES pH 7.6, 5 mM MgCl2, 1 mM EGTA
>
> -> Add 10mM DTT from 1M stock in H2O immediately before use.
>
> :10 ul 1M DTT + 990 ul M5B
>
> #High salt buffer (HSB) + 10 mM DTT
>
> HSB: 500 mM KCl, 10 mM HEPES pH 7.0, 5 mM MgCl2
>
> -> Add 10mM DTT from 1M stock in H2O immediately before use.
>
> :5 ul 1M DTT + 495 ul HSB
>
> #NEM myosin II
>
> Every NEM myosin stock* (in 50% glycerol, functional stored at -20*C indefinitely) should be independently tested to determine appropriate concentration for actin binding in TIRF assay.
>
> -> Dilute to working concentration in HSB+DTT immediately prior to use.
>
> :1 ul NEM myosin + 9 ul HSB+DTT
>
> #250 nM F-actin*
>
> -> Dilute 1 uM phalloidin-stabilized F-actin (should be no older than 3 weeks) in M5B+DTT
> -> always cut the end of the pipette tip using CLEAN scissors or
> razor, to avoid shearing actin

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> :25ul 1uM actin + 75ul M5B+DTT
>
> #BSA 2mg/mL
>
> -> Dilute from 10-100mg/mL BSA in M5B into M5B+DTT
> -> Stock BSA can be used for a few months, until it has accumulated fleuorescent particles from repeated use that makes it unsuitable for TIRF
>
> :10 ul 20 mg/mL BSA + 90 ul M5B+DTT
>
>
> #Motility Assay Buffer
> 10nM-10mM ATP (depending upon application)
> 100mM DTT (this was determined to be ideal for resisting photobleaching of rhodamine without leading to blinking, but can be lowered to ~10mM is this is not a concern)
> 100ug/mL calmodulin* (stocks stored at -20*C for up to 6 months, or -80*C indefinitely)
> optional:
> for ATP regeneration:
> 100ug/mL creatine phosphokinase (diluted from 1mg/mL CPK made daily from powdered stock stored at -20*C indefinitely)
> 1mM phosphocreatine (diluted from 100mM PC pH 7.00, stored at -20*C
> indefinitely)
>
> for photostability of dyes or quantum dots:
> 1:100 dilution of superdeoxy* (prepared fresh biweekly)
> 0.4% glucose (diluted from 40% glucose in M5B, prepared every 2
> months)
>
> 1-100nM myosin,depending on application
> M5B+DTT to final volume
>
> example:
> 1 uL 1mM ATP
> 10 uL 1M DTT
> 0.66 ul 15.5 mg/mL calmodulin
> (10 ul 1mg/mL CPK)
> (1 uL 100mM PC pH 7.00)
>
> (1 ul superdeoxy)
> (1 ul 40% glucose)
>
> 2 ul myosin
> M5B+DTT to 100ul total volume
>
> #Quantum dot labeling of biotinylated myosin:
>
> dilute QDs to 20 nM in M5B+DTT (example: 1 ul 2uM QDs + 99 ul M5B+DTT)
> dilute myosin to ~5 nM (this results in a high probability that any

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> moving QD is attached to a single myosin)
>
> immediately(!!!!) prior to use, mix:
> 2 ul QDs + 2 ul 20 mg/ml BSA + 2 ul myosin (in that order or reverse)
>
> add to MAB at 5% total volume
>
> ~Procedure~
>
> Create a flow cell using strips of tape, coverslip, and slide flow in
> 20 ul NEM wait 5 minutes rinse 2X with 20ul HSB+DTT quickly flow in 20
> ul actin (cut tip!!) quickly rinse with 20ul M5B+DTT block with BSA
> (2mg/mL) wait 5 minutes Add 20 ul MAB