Stock solutions:

1) ATP (100mM)
2) Frozen dynein aliquot
3) PCD (100x)
4) PCA (100x)
5) DTT (1M)
6) Axonemes labeled with Cy5 or Cy3
7) Casein (~40 mg/ml)
8) DLB buffer
9) α-GFP- or streptavidin-coated beads (depending on attachment chemistry)

Solutions to make for each experiment:

DLBC (make 150 μL)
1. Casein (2.5mg/mL) >> 10 μL
2. DLB buffer >> 140 μL

AXO Axoneme sol'n (mix well, solns in glycerol)
1. Axo (Cy5) stock >> 1 μL
2. DLB (no casein) >> 6 μL

Motor Solution (dilution variable, depends on prep)
1. Motor stock >> 1 μL
2. DLBC >> serial dilutions to anywhere between 2x and 100x

Stepping buffer (variable, adjust ATP as needed, this

Protocol:

1. Prepare 150μL of DLBC (see 'Solutions to make for each experiment' above)
2. Prepare thy-mo stock dilutions
3. Mix beads stock by pipetting, take out 2 μL into a small tube, sonicate for 5 sec
4. Mix the 2 μL sonicated beads with 2 μL of diluted protein; let sit for 10 min on ice.
5. (Meanwhile) Make the channel slide:
   1. Cover a glass slide with 2x double-sided tape, leaving ~2mm channel
   2. Put on 18mm coverslip, press down with pipette
   3. Rip off the tape
6. Prepare AXO solution and stepping buffer
7. After 10min, add 14μL of DLB and 2 μL of stepping buffer to the motors+beads solution
8. Flow AXO (by gravity)
9. Flow the 20 μL of Motors+Beads+DLB+Stepping buffer solution prepared in step 7
10. Seal ends of slide with nail polish