**Antibody Staining**

**(modified from Waddle et al, 1994) edited 3/5/07**

1) Cut ~40 young adults into a **~30uL droplet of M9** on subbed slide and cover on 18mm by 18mm coverslip with much wicking.

2) Freeze crack for **30 min** on blocks in dry ice.

3) Fix for **10-15 min** in 4%Form/75%MeOH (or -20°C MeOH depending on 1° antibody)

**Fix: Liquid Form Receipe**

75% Methanol 37.5 mls MeOH

4% Formaldehyde 5 mls of 40% Form (EM grade)

0.5X PBS 1.5 mls 10X PBS

0.1mM EDTA 10 ul of 0.5M EDTA

 5 mls ddH2O

 50 mls

4) Wash **1X** in Blocking Buffer (PBSBT\*--1% BSA in PBST\*) for **30 min**

 Do not let fix contact your writing, it will cause the marker to run

5) 1o Antibody in PBSBT\* overnight @ 4°C -or- 1-2hr @ Room temperature

 *Actin at 1:400, Tubulin at 1:100, Dyn-1 at 1:250*

 Wick as much blocking buffer as possible

 Use parafilm “coverslips”

 ~50 ul pre slide

6) Wash **3X** in PBST\*, **5 min each**

7) 2o Antibody--**1:200 in PBSBT**, 1-2 hours RT

 *Alexa 488 is green (brighter)*

 *Alexa 568 is red*

8) Wash **3X** in PBST\***, 5 min each**

9) If using add 1:4000 TOTO-3 for 5 min and wash 1 more time with PBST\*

10) Mount with **8ul of Vectashield** using an 22mm by 22mm coverslip, let dry, and seal with nailpolish.

You must add the items in order or the PBS will precipitate.

**Mix in coplin jar in order below**

**Fix: (From Waddle et al., 1994)**

**--works very well for tubulin/actin (or related proteins)**

75mls MeOH

10mls Formaldehyde

3mls 10X PBS

12mls ddH20

20ul 0.5M EDTA

**Subbing Solution:**

1. Bring 200 ml of ddH2O to 60 C.

2. Add 0.4g gelatin

3. Cool to 40 C.

4. Add 0.04g Chrome Alum

5. Add 1mM azide

6. Add polylysine to 1mg/ml

Put the subbing solution in a coplin jar and keep it at 4C for months. Put the slides in for 30s - and hour, take them out and let them dry in a test tube rack. The slides should keep for a month or so.

**PBS**

**1X (1L) 10X**

8.0g NaCl 80g NaCl

0.2g KCl 2g KCl

1.44g Na2HPO4 14.4g Na2HPO4

0.24g KH2PO4 2.4g KH2PO4

0.5ml 1M Sodium Azide(FV=1mM)

Dissolve in 800mls of ddH2O

Adjust pH to 7.2

Adjust volume to 1L

**PBSBT\* (0.5% BSA & 0.25% Tween 20)**

 0.5g BSA

 0.25g Tween 20\*

 100mls PBS

 50ul 1M Sodium Azide (FV=1mM)

**PBST\* (0.25% Tween 20)**

 0.25g Tween 20\*

 100mls PBS

 50ul 1M Sodium Azide (FV=1mM)

\*Do not add Tween when staining for membrane associated proteins like Dyn-1