



Title: *Quantum Dot Bead Coupling*

No: RTLP-NANO-2

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

Page 1 of 2

•Lab coat	•Sulfo-NHS	•Tween-20	•Pipettes
•Gloves	•Phosphate Buffered	•dH ₂ O	•Pipetteman
•Virus sample	Saline (PBS)	•Microspin filter	•Pipette Aid
•MES buffer	•Microsphere beads	columns	•Pipetteman tips
• BSA	from Molecular Probes	•Stir plate	•Timer
•EDAC	•YB- & RB- Ab conjugate	•0.65mL EP tubes	•Sonicator
•α-G monoclonal or	•37°C H ₂ O bath	•Centrifuge	•Analytical
α-F monoclonal Ab			Balance

Buffers:

Blocking Buffer:

Combine the following and pH 7.4

- 10mM PBS
- 0.5% BSA
- 0.2% Sodium Azide

Hybridization Buffer:

Combine the following and pH 7.4

- 10mM PBS
- 0.02% Tween 20
- 0.1% BSA
- 0.2% Azide

Procedure:

I. Coupling beads with antibodies:

1. Into a 0.65 mL EP tube combine **80 uL** of MES buffer (pH 6.0), **2 uL** of 40 nm yellow-green beads, and **5 uL** of 40 nm red beads for reaction with the red beads
2. **Sonicate** the mixture for 15-20 minutes.

3. Add 7 uL (1mg/ml) of anti-G monoclonal antibody (1mg/ml, clone 131-2G) or 6 uL (1mg/ml) of anti-F monoclonal antibody (1mg/ml clone 131-2A)
4. In this order add 60 ug (as water solution) of Sulfo-NHS followed by 50 ug (as water solution, let EDAC come to room temp before weighing, store in freezer, desiccated if possible) of EDAC.
5. Let reaction proceed with constant stirring at room temperature for 1 hr.
6. Add 50 ug of EDAC again.
7. Let reaction proceed for another hour.
8. Add PBS-BSA-Sodium Azide blocking buffer to block uncoupled sites (add 30 uL for 100 uL reaction volume, for 30 minutes at 37°C)
9. Centrifuge filter using S-300HR columns at 740 at rcf setting on the eppendorf centrifuge. *NOTE: wash column before use with 400-500 uL PBS.*

II. Procedure for Virus - antibody hybridization

1. Into a 0.65 ml EP tube combine the following:
 - 5 uL of YB-Ab conjugate.
 - 5 uL of RB-Ab conjugate.
 - 25 uL or the volume desired of the virus sample
 - 215 uL of PBS-TBN hybridization buffer
2. Let the tubes incubate at 37° C for 2 hours.
3. Dilute 200 times and use for coincidence measurements.