Immunofluorescence.

	Initials	Date
Prepared By	ER	
Approved By		

WILSON JONES COMPANY G7506 ColumnWrite® MADE IN U.S.A

A **Make Slides**

1. Aliquot Cells
   - Microfuge 5000 rpm for 5 mins at R. temp.
   - Aspirate supernatant using a pasteur pipette adapted with a pipette tip
   - add 1 ml of 1X PBS and pipette cells up and down to mix (Do Not Vortex)
   - Aspirate leaving approximately 2 ml of 1X PBS
   - Mix cells by pipetting to uniformly mix cells.
2. Add approx. 1 µl of cells to cells and spread evenly to distribute cells.
   - let cells air dry.
3. Fix in 1:1 Acetone / Methanol at −20°C for 10 mins
4. Air Dry
5. Block Incubate with 20% Goat Serum 30 mins at Room temperature. (Humidity Chamber).
6. Wash with 1X PBS
7. Incubate (Humidity chamber) with Specific antibodies 1. α Bam 2 } 1/200 - 1/500 dilute in PBS 1X
   2. α gp 110
   - incubate 1-2 hrs at room temperature.
8. Wash well with 1X PBS 4-5 5 minute washes.
9. Incubate with Goat α Mouse Ig - FITC 1/1000 - 1/2000
10. Wash well with 1X PBS.
11. Keep Moist.
12. Add Anti Fade 100 µl and cover slip. Flip slide and press firmly to remove any bubbles trapped

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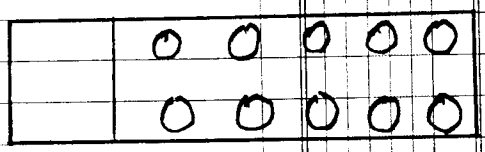
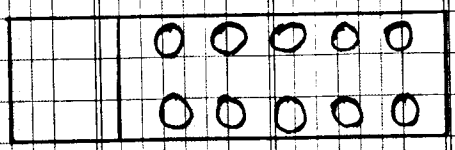


1. Goat Serum
2. α Bam Z or αgp110
3. Goat α Mouse Ig - FITC



1. Goat Serum
2. —
3. Goat α Mouse Ig - FITC

e.g BJAB, BJAB/Bam Z, BJAB/B95-8

BJAB Bam Z

42 + 44 / 2 = 43

$43 \times 2 \times 10^4 = 8.6 \times 10^5 = 860{,}000$ cells/ml

40 µl cells + 40 µl trypan blue = ½ df

	#1	#2	#3	#4
Volume	2.0 ml	1.5 ml	1 ml	0.5 ml
# cells	1.7 mill.	1.3 mill	900,000	500,000 / 20 µl 1x PBS
# cells/µl	85,000	65,000	45,000	25,000 / 1 µl 1x PBS