**ANTIBODY DATA SHEET**

**GENERAL INFORMATION**

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| NAME : | 5F2B3 – C8 (obtained 07/2007) |
| CLONALITY : | Monoclonal |
| SPECIES OF ORIGINE : | Mouse |
| ANTIBODY SUBCLASS : | IgM |
| ANTIBODY SPECIFICITY : | Anti BILBO1 (recognizes an epitope within the coiled-coil domain) |
| IMMUNOGEN : | 6His-BILBO1 |
| STATUS : | Cell culture supernatant containing 0,02 % sodium azide as preservative  Cell line in Liquid nitrogen |
| STORAGE : | Store supernatant at +4°C indefinitely |
| OBTAINED FROM : | D. Robinson, home made |

**USAGE**

**Works on cytoskeletons, not whole cells.**

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| Immunofluorescence | Fix cytoskeletons at -20°C in methanol 30 min (or longer), or with 3% Paraformaldehyde in PBS for 10 min and neutralyze PFA with 100 mM glycine for 5 min (2x) then wash in PBS.  Dilute the antibody 1:10 in PBS. Incubate 1hr. |
| Western blotting | We usually load 5.106 cytoskeletons (on a Biorad SDS-PAGE, 1.5 mm thick, 10 wells) and use the antibody diluted1:10 in TBS + 5 % Milk + 0,2% Tween20, overnight at 4°C. |
| Immunogold | - |

**REFERENCES**

**Bonhivers M, Nowacki S, Landrein N, Robinson DR.** [Biogenesis of the trypanosome endo-exocytotic organelle is cytoskeleton mediated.](http://www.ncbi.nlm.nih.gov/pubmed/18462016)**PLoS Biol.** 2008 May 6;6(5):e105

**IFA Anti-Bilbo1 (5F2B3-C8) Mouse monoclonal IgM.**

This protocol is designed for *T. brucei brucei* 427 29.13 procyclic form cells using multi-well slides.

**Cell fixation:**

Cells can be harvested by centrifugation 1,000 x g and washed by resuspending in an equal volume of PBS (pH 7.0).

A second wash step may be needed and this can be followed by resuspending in 1/5th to 1/10th the original volume of cells.

PCF : they stick to poly-l-lysine coated slides

BSF: they don’t stick well and tend to detach. So, what we do is to pellet the cells and resuspend them in a small volume of extraction solution and load immediately on poly-l-lysine coated slide.

Cells should be gently settled on Poly-L-Lysine coated slides for 5 minutes (25µL/well).

**Extraction cells :**

Extract for 5 minutes with a 25-50µL drop per well of 0.25% NP40 in 100mM PIPES, 1mM MgCl2. (you can also use 1% instead of 0.25% NP40 as for most people in the lab, 1% is better).

A second extraction may be needed for resistant cells or a large number of cells.

Wash 2-3 x 5 minutes in 100mM PIPES, 1mM MgCl2 (50μL/well).

Fix by immersion in pre-cooled -20°C Methanol for at least 30 minutes and rehydrate/Wash 2 x 5 minutes in PBS (25-50µL/well). Or fix in 3% Paraformaldehyde (in PBS) for 10 min and neutralize with 100 mM glycine for 5 min (2x) then wash in PBS.

**Note : Anti-Bilbo1 (5F2B3-C8) does not work on non-extracted cells (whole cells):**

Incubate Anti-Bilbo1 antibody (1 hour at room temp) in a moist chamber (25-50µL/well).

This batch of Anti-Bilbo1 clone 5F2B3-C8 can be used diluted 1:5 to 1:10 in PBS. (It is a Mouse monoclonal IgM).

After incubation of Anti-Bilbo1, wash 2 times 5 minutes in 50 μL PBS per well.

Incubate with your secondary antibody for 1 hour at Room temp (Anti mouse IgM specific or IgG whole molecule (H+L)).

After secondary antibody incubation, wash the slide 2 times 5 minutes in 50 μL PBS.

Add DAPI : 25 μL of 10 μg/mL in PBS for 3 minutes.

Mounting/Antifading solution: we use Slowfade Molecular Probes #S-36936

Add a cover slip and seal the coverslip.