**Contract ZFR13028  
MmSAXO1**

**(aa 144-421)**

**Hen H011 (Preimmune PPI HS058) and H012 (Preimmune PPI HS061)**

**Pre-immune were tested by Benoit Roger (date 2013 02 22) by immunofluorescence on U-2 OS non transfected cells.**

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| **Hen H011** | | | **H012** | | |
|  | Immuno-Fluorescence | Western-Blot |  | Immuno-Fluorescence | Western-Blot |
| Collection 1 week 1 (C1W1) (17-23/04/2013) | U-2 OS MmSAXO1-GFP transfected cells:  -1:100. Positive signal but very high background. Needs more dilutions. Centriole is very difficult to see. I can see some MT not visible with GFP labelling?  **Positive. Test higher dilutions (up to 1:15000).**  **Test MetOH fix for centriole labelling.**  **mouse sperm (C57BL/6)**  **Methanol fixation**, PreI, W1 and W2 dilutions 1:50 1:100  1:2001:400;1:800;1:1600;1:3200;1:6400;1:12800 1:25600 1:51200 1:102400  PreI everywhere but weak and negative at 1:400 and more  W1 and W2 bright signal only on the flagella, starting AFTER intermediate piece to the tip. Best W2 at 1:400 and 1:800. No signal on centriole. Similar to H012  **Positive. Test with classic protocol used before (PFA and Citrate buffer bath at 98°C) for better extraction?**  **mouse sperm (C57BL/6)**  **PFA fixation+ Citrate buffer (98°C),** **(before or after PFA),** PreI, W1 and W2 dilutions 1:50 1:100  1:2001:400;1:800;1:1600;1:3200;1:6400    PreI: everywhere but weak and negative at 1:400 and more.  W1 and W2 bright signal only on the flagella, including intermediate piece to the tip. Best W2 at 1:400 and 1:800. No signal on centriole. Dissociated MT (3 or 5) are present at the tip and each has on dot at the end.  Similar to H012  **Positive. Better extraction but never labelling on the centriole** | **Tested on purified His-tagged Proteins (produced for immunization). 500ng of purified prot. Antibody diluted 1:5000, overnight at 4°c, 1M NaCl wash, anti-chicken HRP 1:10000 for 1hour. ECL + DAB**  **Positive signal using ECL and DAB with no signal using pre-immune.**  **Test with bacterial extract and other His-tagged protein…** | Collection 1 week 1 (C1W1) (17-23/04/2013) | U-2 OS MmSAXO1-GFP transfected cells:  **Very similar to hen H011 but maybe a bit more specific than H011?. Still centriole not visible.**  Tested again at higher dilutions (1:2000 and 1:10000)  With PFA fixation.   * 1:2000. Transfected cells are positive. Still high background. Centrosomes not labelled in red when visible in green. * 1:10000 low expression cells hard to detect.   PreI everywhere but weak and negative at 1:400 and more  W1 and W2 bright signal only on the flagella, starting AFTER intermediate piece to the tip. Best W2 at 1:400 and 1:800. No signal on centriole  **Positive. Test with classic protocol used before (PFA and Citrate buffer bath at 98°C) for better extraction?** Similar to H011  **mouse sperm (C57BL/6)**  **PFA fixation+ Citrate buffer (98°C) (before or after PFA),,** PreI, W1 and W2 dilutions 1:50 1:100  1:2001:400;1:800;1:1600;1:3200;1:6400    PreI: everywhere but weak and negative at 1:400 and more.  W1 and W2 bright signal only on the flagella, including intermediate piece to the tip. Best at 1:400 and 1:800. No signal on centriole. Dissociated MT (3 or 5) are present at the tip and each has on dot at the end.  Similar to H011  **Positive. Better extraction but never labelling on the centriole** | Same as hen H011 |
| Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS MmSAXO1-GFP transfected cells:  **Same as C1W1.**  Mouse sperm **PFA fixation+ Citrate buffer (98°C), or Methanol**  **same as C1W2** | Mouse sperm (C57BL/6) on 1e4 spz and testis 50ug Testis with PI 1:1000, W2 1:1000;1:3000,1:9000, wash NaCl 1M revelation ECL DAB  **On sperm:**  **All Positive but two bands close together (around 54 kDa) one of them could be SAXO1. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative**  **On testis:**  **Several bands not present on PreI (between 20 to 60 kDa). Not the same bands and weaker than for those of H012 Negative?** | Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS MmSAXO1-GFP transfected cells:  **Very similar to hen H011 but maybe a bit more specific than H011?. Still centriole not visible.**  Mouse sperm **PFA fixation+ Citrate buffer (98°C), or Methanol**  **same as C1W2**  **ImmunoHisto mouse testis biopsy 13-03-2013** (spz have been see):  **Methanol fixation +citrate Buffer**  **OR**  **PFA fixation + citrate Buffer**  Dilution 1:50, 1:100, 1:200, 1:400, 1:800  PI and W2  AceTub 1:1000  **Negative** weak signal everywhere no specific (no difference with PI) even with many spz were present and labelled with aceTub. No co labelling | Mouse sperm (C57BL/6) on 1e4 spz and testis 50ug Testis with PI 1:1000, W2 1:1000;1:3000,1:9000, wash NaCl 1M revelation ECL DAB  **On sperm:**  **All Positive, one specific band (around 54 kDa). Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative**  **On testis:**  **Several strong bands not present on PreI (between 20 to 60 kDa). Not the same bands found for H011. Negative?** |

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| **Collections 1**  **week1+2 (C1,W1+W2)**  **- Collections 2**  **week1+2 (C2,W1+W2)**  **- Collections 3**  **week1+2 (C3,W1+W2)** |  | Mouse sperm (already tested with FAM154A) on 5e6 spz with PI 1:1000, w1+2 1:1000;1:3000, wash NaCl 1M revelation ECL DAB  **On sperm: Positive (?)**  **for collection1 Positive but two bands close together (around 54 / 60kDa) one of them could be SAXO1. And one band at 22 kDa. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **for collection2 one Positive band (around 54 / 60kDa). And one band at 22 kDa. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **for collection3 one Positive band (around 54 / 60kDa). And one band at 22 kDa. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **The best C3 in 1:3000** |  |  | Mouse sperm (already tested with FAM154A) on 5e6 spz with PI 1:1000, w1+2 1:1000;1:3000, wash NaCl 1M revelation ECL DAB  **On sperm: Positive**  **For all collection: one strong band at 54 / 60 kDa with one at 22 kDa.**  **The best C3 in 1:3000** |
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