**Contract ZFR13027  
HsSAXO2**

**(aa 162-355)**

**Hen H009 (Preimmune PPI HS056) and H010 (Preimmune PPI HS057)**

**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 H009** | | | **H010** | | |
|  | Immuno-Fluorescence | Western-Blot |  | Immuno-Fluorescence | Western-Blot |
| Collection 1 week 1 (C1W1) (17-23/04/2013) | U-2 OS HsSAXO2-Myc transfected cells:  -Myc signal is whole cell and sometimes nucleus.  -1:100: positive but needs higher dilution. Nucleus labelling not really visible.  -1:1000: needs higher dilutions.  **Positive. Test more dilutions.**  **Test MetOH for nucleus labelling.**  Tested again at higher dilutions (1:2000 and 1:10000)  With PFA fixation. Same as hen H010.  Human sperm n° 139 and 141, methanol fixation, serum PI, w1 and w2  Dilutions tested: 1:25;1:50; 1:100;1:200;1:400;1:800  **Negative labelling: No specific labelling compared to PI**  Human sperm n° 147 methanol fixation and PFA 3%, serum PI, w1 and w2  Dilutions tested: 1:400;1:800; 1:1600;1:3200;1:6400;1:12800  **Positive methanol**  **W1 and W2 1:3200 strong centriole and along flagella**  **(PI negative)** | **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 HsSAXO2-Myc transfected cells:  **Very similar to H009 but H010 a bit more specific?**  Tested again at higher dilutions (1:2000 and 1:10000)  With PFA fixation.   * 1:2000. Transfected cells are positive. No particular localization though. Same signal in green (myc) and red. * 1:10000 low expression cells hard to detect.   Human sperm n° 139 and 141, methanol fixation, serum PI, w1 and w2  Dilutions tested: 1:25;1:50; 1:100;1:200;1:400;1:800  **Negative: No labelling compared to PI**  Human sperm n° 147 methanol fixation and PFA 3%, serum PI, w1 and w2  Dilutions tested: 1:400;1:800; 1:1600;1:3200;1:6400;1:12800  **Negative labelling** | Same as hen H009 |
| Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS HsSAXO2-Myc transfected cells:  **Same as C1W1**  Human sperm n° 139 & 141  **Same as C1W2** |  | Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS HsSAXO2-Myc transfected cells:  **Very similar to H009 but H010 a bit more specific?**  Human sperm n° 139 & 141  **Same as C1W2** |  |

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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)** | Human sperm n° 147 **methanol** fixation serum PI, w1 + w2  Dilutions tested: 1:200;1:400;1:800; 1:1600;1:3200;1:6400  **No specific (???) for collection 1, 2, 3**  1:200;1:400; Strong signal along flagella and at the base of the head (Neck but not centriolar!) decrease after 1:800 | Human sperm n° 110 (already tested with FAM154A) on 5e6 spz with PI 1:1000, w1+2 1:1000;1:3000, wash NaCl 1M revelation ECL DAB  **All strong signal even in 1:3000, several bands (not present with the PI) between 30 to 100kDa.** |  | Human sperm n° 147 **methanol** fixation serum PI, w1 + w2  Dilutions tested: 1:200;1:400;1:800; 1:1600;1:3200;1:6400  **No specific (???) for collection 1, 2, 3**  1:200;1:400; Strong signal along flagella and at the base of the head (Neck but not centriolar!) decrease after 1:800 | Human sperm n° 110 (already tested with FAM154A) on 5e6 spz with PI 1:1000, w1+2 1:1000;1:3000, wash NaCl 1M revelation ECL DAB  **All strong signal even in 1:3000, several bands (not present with the PI) between 30 to 100kDa** |
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