**Contract ZFR13016  
Nter-HsSAXO1**

**(4x aa 1-66)**

**Hen H005 (Preimmune PPI HS053) and H006 (Preimmune PPI HS054)**

**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 H005** | | | **H006** | | |
|  | Immuno-Fluorescence | Western-Blot |  | Immuno-Fluorescence | Western-Blot |
| Collection 1 week 1 (C1W1) (17-23/04/2013) | U-2 OS HsSAXO1-GFP transfected cells:  - 1:100. Negative. Crap. Very strong Background (BG) (no first control had low BG). Even on high SAXO1-GFP expressing cells, I did not see a specific signal.  - 1:1000: same as 1:100.  **Not positive BUT Test higher dilutions (2000, 3000, 4000, 5000?).**  **Test MetOH fixation.**  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  **Stick everywhere, high background on PI and immune w1 and w2. Perhaps centriole?**  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  **PFA negative**  **Methanol :**  **PI labelled Flagella and centriole weakly but different from w1 and w2 with 1 or 2 dots in centrioles.**  **W1 1:600 mainly centriole, weak flagella (positive)**  **W2 1:800 mainly centriole and weak flagella (positive)** | **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 HsSAXO1-GFP transfected cells:  **same as hen H005**  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  **Centriole + along flagella with 1:800 (PI strong)**  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  **PFA negative**  **Methanol : Not positive**  **weak no really different between W1&2 and PI** | **same as hen H005** |
| Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS HsSAXO1-GFP transfected cells:  **- same as C1W1**  Tested again at higher dilutions (1:2000 and 1:10000)  With PFA fixation.   * 1:2000. Strong background still. No red signal on GFP positive cells. * 1:10000 less background. No specific signal.   Human sperm n° 139 &141  **same as C1W1** | Human sperm n° 109 (already tested with FAM154A) on 5e6 spz with PI 1:100, w2 1:100;1:1000, wash BS  **Positive Weak band at 54 kDa (absent in PI)**  Human sperm n° 110 (already tested with FAM154A) on 5e6 spz with PI 1:1000, w2 1:1000;1:3000,1:9000, wash NaCl 1M revelation ECL DAB  **All Positive and clean with ECL (10 sec) and DAB (best 1:3000) and better than chicken H006** | Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS HsSAXO1-GFP transfected cells:  **- same as hen H005**  Human sperm n° 139 &141  **same as C1W1** | Human sperm n° 109 (already tested with FAM154A) on 5e6 spz with PI 1:100, w2 1:100;1:1000, wash BS  **Positive Weak band at 54 kDa (absent in PI)**  Human sperm n° 110 (already tested with FAM154A) on 5e6 spz with PI 1:1000, w2 1:1000;1:3000,1:9000, wash NaCl 1M revelation ECL DAB  **All Positive and clean with ECL (10 sec) and DAB (best 1:9000)** |
| 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  **Negative for collection 1, 2, 3**  (no centriolar, no flagellar) (PI and head labelling) | 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  **C1 and C2 Positive (one main band at 50-60 kDa) and quite clean with ECL (10 sec) and DAB (best 1:3000). (C3 others bands appear with same intensity)** |  | Human sperm n° 147 **methanol** fixation serum PI, w1 + w2  Dilutions tested: 1:200;1:400;1:800; 1:1600;1:3200;1:6400  **Negatif** (no signal) | 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  **C1 C2 C3 Positive (one main band at 50-60 kDa) and quite clean with ECL (10 sec) and DAB (best 1:3000).** |
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