**Contract ZFR13026  
HsSAXO1**

**(aa 193-417)**

**Hen H007 (Preimmune PPI HS048) and H008 (Preimmune PPI HS051)**

**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 H007** | | | **H008** | | |
|  | 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: maybe positive signal on high expressing cells but very high background.  - 1:1000: positive on high expressing cells. Still strong background. Very difficult to see the centrioles.  **Positive. Test MetOH fix (to see the centrioles)**  **Test higher dilution.**  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  **Positive: 2 dots on centrioles + flagella, best 1:800**  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**  **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 HsSAXO1-GFP transfected cells:  **Same as hen H007**  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  **Positve : 2 dots on centrioles + flagella, best 1:800**  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  **Perhaps Positive methanol?**  **For W2 with 1:3200 weak centriole and flagella**  **(and sometimes head of spz)** | Same as hen H007 |
| Collection 1 week 2 (C1W2) | 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. No cells on the coverslip ?? * 1:10000 High expressing cells only are positive with antibody. Centrosome not labelled although visible in GFP …   Human sperm n° 139 &141  **same as C1W1**  **ImmunoHisto human testis biopsy N**°24 19-03-2012(spz have been see):  PFA+ citrate buffer  Dilution 1:800, 1:1600, 1:3200, 1:6400, AceTub 1:1000  PI and W2  **Negative**  No colabelling with aceTub, weak signal everywhere no specific (no difference with PI)  **ImmunoHisto human kidney biopsy** 24-05-2011  PFA+ citrate buffer  Dilution 1:800, 1:1600, 1:3200, 1:6400, AceTub 1:1000  PI and W2  **Negative**  Many primary cilia are labelled with aceTub but not with chicken, no centriole | Human sperm n° 109 (already tested with FAM154A) on 5e6 spz with PI 1:100, w2 1:100;1:1000, wash BS  **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 strong Positive and clean with ECL (4 sec) and DAB (best 1:3000 1: 9000) (similar to chicken H008)** | Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS HsSAXO1-GFP transfected cells:  **Same as hen H007**  Tested again at higher dilutions (1:2000 and 1:10000)  With PFA fixation.   * 1:2000. Positive. Centrosomes not labelled although visible with GFP. Low expressing cells hard to detect. Background quite high still. * 1:10000 Signal/noise ratio much better but high expressing cells only are positive with antibody. Centrosome not labelled although visible in GFP   Human sperm n° 139 &141  **same as C1W1**  **ImmunoHisto human testis biopsy N**°24 19-03-2012(spz have been see):  PFA+ citrate buffer  Dilution 1:800, 1:1600, 1:3200, AceTub 1:1000  PI and W2  **Negative**  No co labelling with aceTub, weak signal everywhere not specific (no difference with PI) | Human sperm n° 109 (already tested with FAM154A) on 5e6 spz with PI 1:100, w2 1:100;1:1000, wash BS  **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 strong Positive and clean with ECL (4 sec) and DAB (best 1:3000 1: 9000) (similar to chicken H008)** |

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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  **Negative for collection 1, 2, 3**  (1:200;1:400 Strong signal but not centriolar, not flagellar, marginal zone and everywhere labelling after 1:800 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  **All strong Positive and clean with ECL (4 sec) and DAB (best 1:3000) (similar to chicken H008)** |  | 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**  (1:200;1:400 Strong signal but not centriolar, not flagellar, marginal zone and everywhere labelling after 1:800 no signal) idem H007 | 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 Positive and clean with ECL (4 sec) and DAB (best 1:3000) (similar to chicken H007)** |
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