**Contract ZFR13029  
MmSAXO2**

**(aa 185-353)**

**Hen H013 (Preimmune PPI HS063) and H014 (Preimmune PPI HS064)**

**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 H013** | | | **H014** | | |
|  | Immuno-Fluorescence | Western-Blot |  | Immuno-Fluorescence | Western-Blot |
| Collection 1 week 1 (C1W1) (17-23/04/2013) | U-2 OS MmSAXO2-GFP transfected cells:  **No conclusion because no MmSAXO2-GFP expression.**  Tested again in same conditions. No transfected cells again… Try with another plasmid.  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 no specific signal compare to PreI, everywhere but weak.  **Negative. 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 no specific signal compare to PreI, everywhere but weak and strongly at the acrosome.  **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 MmSAXO2-GFP transfected cells:  **No conclusion because no MmSAXO2-GFP expression.**  Tested again in same conditions. No transfected cells again… Try with another plasmid.  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 no specific signal compare to PreI, everywhere but weak.  **Negative. 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 no specific signal compare to PreI, everywhere but weak and strongly at the acrosome.  **Negative.** | Same as hen H013 |
| Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS MmSAXO2-GFP transfected cells:  **No conclusion because no MmSAXO2-GFP expression.**  Mouse sperm **PFA fixation+ Citrate buffer (98°C), or Methanol**  **same as C1W** | 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:**  **negative**  **On testis:**  **Several bands not present on PreI (between 25 to 40 kDa). Strong band at 30 kDa. Same bands found with H014. Positive?** | Collection 1 week 2 (C1W2) (24-30/04/2013) | U-2 OS MmSAXO2-GFP transfected cells:  **No conclusion because no MmSAXO2-GFP expression.**  Mouse sperm  **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:**  **negative**  **On testis:**  **Several bands not present on PreI (between 25 to 40 kDa). Strong band at 30 kDa. Same bands found with H013. Positive?** |

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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 several bands (around 54 / 60kDa) one very dense of them could be MmSAXO2. And one band at 30 kDa. Revelation with ECL (30 sec) and DAB (best 1:1000) and PI negative.**  **for collection2 Positive but several bands (around 54 / 60kDa) one very very dense of them could be MmSAXO2. And one band at 30 kDa. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **for collection3 one bright Positive band (around 54 / 60kDa). Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **The best C3 in 1:3000**  **(Better than H014)** |  |  | 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 some weak bands around (around 54 / 60kDa) and 30kDa Revelation with ECL (30 sec) and DAB (best 1:1000) and PI negative.**  **Not very good**  **for collection2 Positive (?) but several bands (around 54 / 60kDa) one of them could be MmSAXO2. And one band at 30 kDa. Revelation with ECL (30 sec) and DAB (best 1:3000) and PI negative.**  **for collection3 idem collection 2**  **Used H013 (better than H014)** |
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