Testing the blocking efficacy of SophoMer F10 and BSA in immunohistochemistry on paraffine embedded control mouse testes section

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Objective

To evaluate the performance of SophoMer F10 as a blocking agent in immunohistochemical staining of paraffin-embedded mouse tissue sections, we compared its efficacy with a conventional multi-component blocking solution consisting of **3% BSA supplemented with 3% horse serum and 3% goat serum**. SophoMer F10 used at **0.4%** provided blocking efficiency comparable to the standard BSA/serum-based block, despite being applied at a substantially lower concentration and with a simplified composition.

Materials and Methods

Fixed in modified Davidson's fluid for 24 h-fixed, paraffin-embedded mouse testes sections (4 μm) were deparaffinized in xylene and rehydrated through graded ethanol series. Heat-induced antigen retrieval was performed in 0.01M Sodium citrate buffer (pH 6) using a pressure cooker at **70 kPa for 15 minutes**. Sections were subsequently permeabilized with **0.2% Triton X-100 in TBS** for 15 minutes.

Non-specific antibody binding was blocked using either **3% BSA supplemented with 3% horse serum and 3% goat serum** or **0.4% SophoMer F10**, both prepared in **TBS containing 0.1% Tween-20**, for 1 hour at room temperature. Primary antibodies, diluted in the corresponding blocking solution, were applied overnight at 4 °C in a humidified chamber. Sections were then incubated with fluorescently labeled secondary antibodies for 2 hours at room temperature.

Finally, sections were mounted using ProLong Gold Antifade mounting medium (Thermo Fisher Scientific), and images were acquired as Z-stack images using a Dragonfly fluorescence microscope equipped with Zyla 4.2 PLUS sCMOS 2048x2048 and a 40x/1.25 objective.

Results

The anti-FBXO38 antibody specifically labeled positive cells with comparable signal intensity and background levels under both blocking conditions, with equivalent performance observed using single-component SophoMer F10 and the multi-component blocking solution containing 3% BSA supplemented with 3% horse serum and 3% goat serum. (see Figure 1 for details).

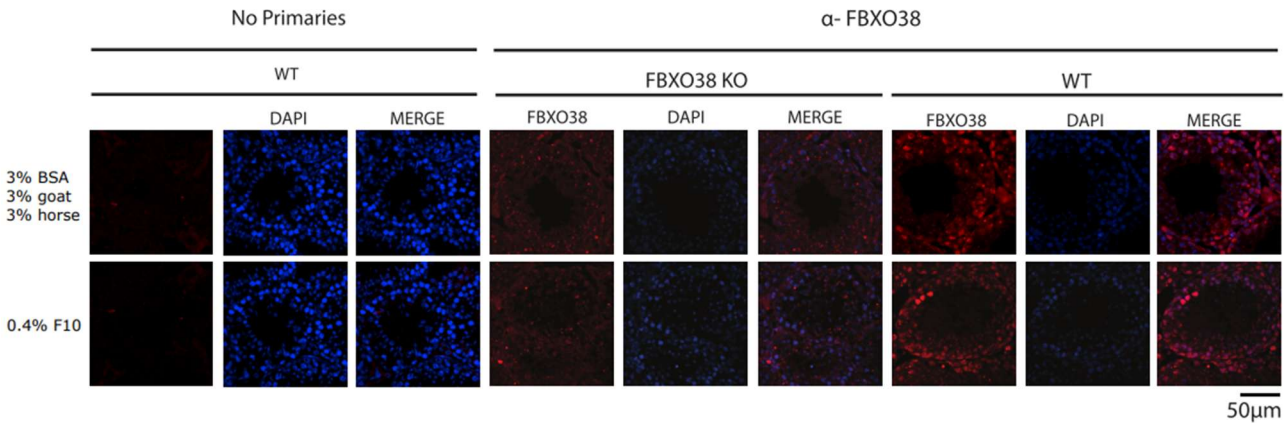

Figure 1 - Representative immunofluorescence images of paraffin-embedded mouse testis sections stained for FBXO38 (red) and counterstained with DAPI (blue). Sections were blocked using either 0.4% SophoMer F10 or a conventional blocking solution containing 3% BSA supplemented with 3% horse serum and 3% goat serum. No-primary antibody controls are shown for wild-type (WT) tissue. FBXO38 staining is shown in FBXO38 knockout (KO) and WT testes following incubation with anti-FBXO38 antibody. Merged images are shown for each condition.