

## Supplemental Material: [10.1177/0300985815626573](https://doi.org/10.1177/0300985815626573)

### Western blot analysis

#### MDM2 western blot analysis

To test the specificity of anti-MDM2 antibody for canine tissue a western blot analysis was performed. Fresh tissue specimens from normal testes (100 mg) were lysed in 1ml of SDS buffer (50mM Tris-HCl pH 6.8; 2% SDS; 5% glycerol) supplemented with 10 µl of protease inhibitor cocktail (P8340, Sigma-Aldrich, Co, St Louis, MO, USA). Lysate protein content was determined by a Protein Assay Kit (TP0300, Sigma). Aliquots containing 10 µg and 30 µg of total protein were separated on NuPage 4-12% bis-Tris Gel (Life technologies-Invitrogen) for 45 min at 200V. Proteins were then electrophoretically transferred onto a nitrocellulose membrane. Blots were washed in PBS and protein transfer was checked by staining the nitrocellulose membranes with 0.3% Ponceau Red. Non-specific binding on nitrocellulose membranes was blocked with 5% milk powder in PBS-T20 (phosphate buffer saline-0.1% tween-20) for 1 h at room temperature. The membranes were then incubated overnight at 4°C with anti-MDM2 mouse monoclonal antibody (mouse monoclonal, clone 2A10, Abcam, dilution 1:100) diluted in Tris buffered saline-T20 (TBS-T20 20mM Tris-HCl, pH 7.4, 500 mM NaCl, 0.1% T-20). After washings with PBS-T20, the membranes were incubated with the secondary biotin-conjugated antibody and then treated with a 1:1000 dilution of an anti-biotin horseradish peroxidase (HRP)-linked antibody. The western blots were developed using a chemiluminescent substrate (Super Signal West Pico Chemiluminescent Substrate, Pierce Biotechnology, Inc, Rockford, IL, USA) according to the manufacturer's instructions. The luminescent signal intensity of the resultant bands was determined by Fluor-STM Multimager using the Quantity One Software (Bio-RAD).

### Results:

Western blot analysis for MDM2 on normal canine testicular tissue revealed a band with the 90kDa expected molecular weight, indicating cross-reactivity of the clone 2A10 with the canine molecule (Supplemental figure 1).