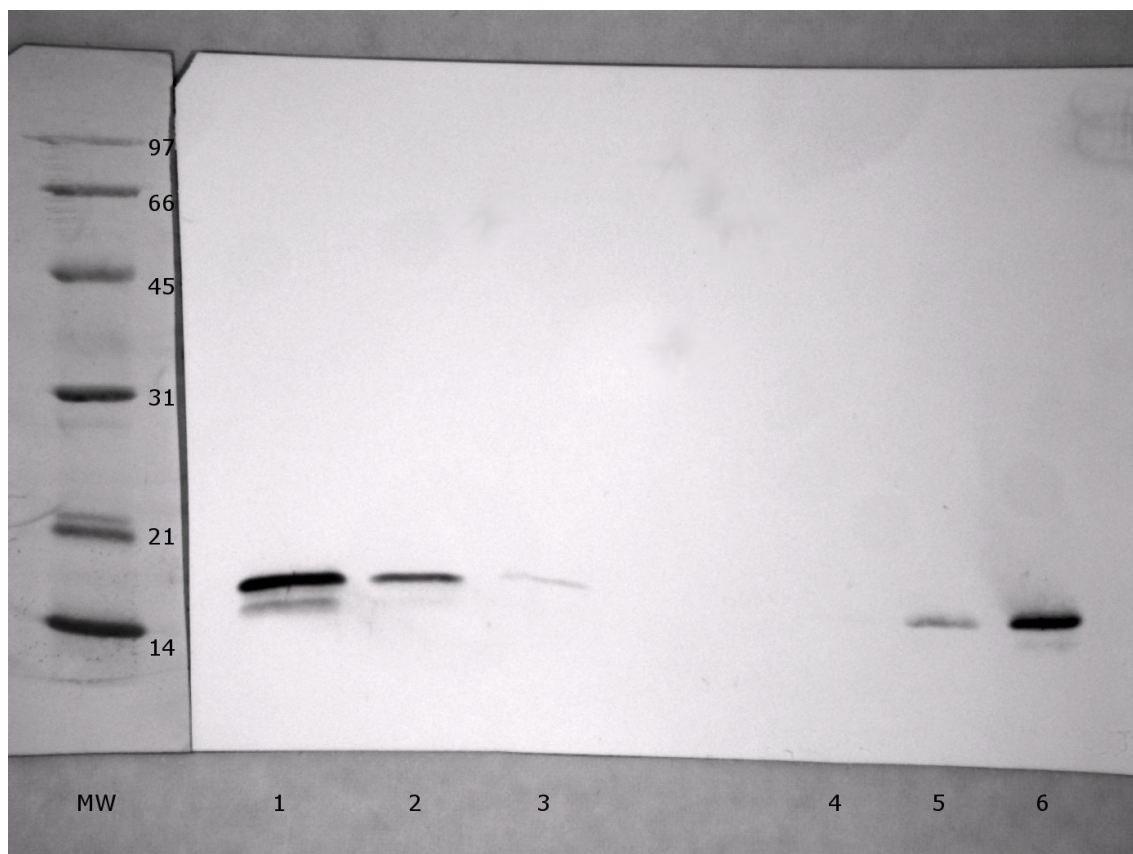


WESTERN BLOT PROTOCOL

Cystatin C Canine, Rabbit Polyclonal Antibody

Cat. No.: RD481009100

Western Blot staining of a control protein



MW Marker.: MW: 97, 66, 45, 31, 21, 14 kDa, (Bio-Rad, USA),

Lane 1:	Recombinant protein	10 ng/Lane, Reducing
Lane 2:	Recombinant protein	1 ng/Lane, Reducing
Lane 3:	Recombinant protein	0.1 ng/Lane, Reducing
Lane 4:	Recombinant protein	0.1 ng/Lane, Non-reducing
Lane 5:	Recombinant protein	1 ng/Lane, Non-reducing
Lane 6:	Recombinant protein	10 ng/Lane, Non-reducing

Recombinant protein Cystatin C Canine E. coli (RD472009100) was subjected to SDS PAGE followed by Western Blot with Cystatin C Canine, Rabbit Polyconal Antibody (RD481009100) at a concentration of 1 µg/ml. Stained with DAB.

1. ELFO:

Polyacrylamide gel electrophoresis (PAGE) was used according to the method of Laemmli with minor modifications.

Slab gels (6 x 8 cm), 1 mm thick, were prepared in a multiple gel casting modul (Mini PROTEAN® 3 System, Bio-Rad, USA).

Stacking gel:

4% acrylamide was prepared from a stock solution of 40% acrylamide/bis-acrylamide, 37.5:1 (Bio-Rad, USA) and diluted with 0.8 M Tris (pH 6.8); SDS was added to the final concentration of 0.1%.

Separation gel:

12% polyacrylamide prepared from a stock solution of 40% acrylamide/bis-acrylamide, 37.5:1 (Bio-Rad, USA) and diluted with 1.5 M Tris (pH 8.8); SDS was added to the final concentration of 0.1%.

Polymerisation was achieved with 0.1% v/v N'N'N N-tetramethyl ethylenediamine (TEMED) and 0.1% ammonium persulphate.

Sample preparation:

The protein concentration was determined by the BCA method (with Bovine Albumin as a standard).

Nonreducing conditions:

Protein samples were mixed 1:1 with nonreducing sample buffer (0.19 M Tris, 2% SDS, 1% (v/v) glycerol and 0.001% Bromophenol blue)

Reducing conditions:

Protein samples were mixed 1:1 with reducing sample buffer (0.19 M Tris, 2% SDS, 1% (v/v) glycerol, 0.001% (w/v) Bromophenol blue and 5% 2-Mercaptoethanol) and boiled for 6 min.

Gels were run at 100 V for 20 min and than at 200 V for 50 min.

Running Buffer: 0.025 M Tris, 0.192 M glycine and SDS 0.1%, pH 8.3.

2. WESTERN BLOT:

SDS-PAGE separated proteins were blotted onto the PVDF membrane at 500 mA for 15 minutes at RT.

Transfer buffer for semidry blotting:

20% methanol, 0.0125 M Tris, 0.096 M glycine and SDS 0.05%.

Membrane with transfered protein was blocked in a blocking buffer for 1 hour at RT.

Blocking buffer:

0.05 M Tris, 0.15 M NaCl, 0.05% Tween, 0.05% Gelatine, 0.02% Thimerosal

3. DETECTION:

Detection of a recombinant protein Cystatin C Canine E. coli (RD472009100) (BioVendor – Laboratorní medicína a.s., Czech Republic)

Primary antibody:

Cystatin C Canine, Rabbit Polyclonal Antibody (RD481009100) – concentration 1µg/ml in 0.05 M Tris, 0.15 M NaCl, 0.05% Tween, 0.05% Gelatine, 0.02% Thimerosal

Incubation: 1 hour

Washing: 3x in 0.05 M Tris, 0.15 M NaCl, 0.05% Tween

Secondary antibody:

Anti-Rabbit HRP-Conjugate (DAKO) – 1: 2000 in 0.05 M Tris, 0.15 M NaCl, 0.05% Tween, 0.05% Gelatine, 0.02% Thimerosal

Incubation: 1 hour

Washing: 3x in 0.05 M Tris, 0.15 M NaCl, 0.05% Tween

Substrate: DAB