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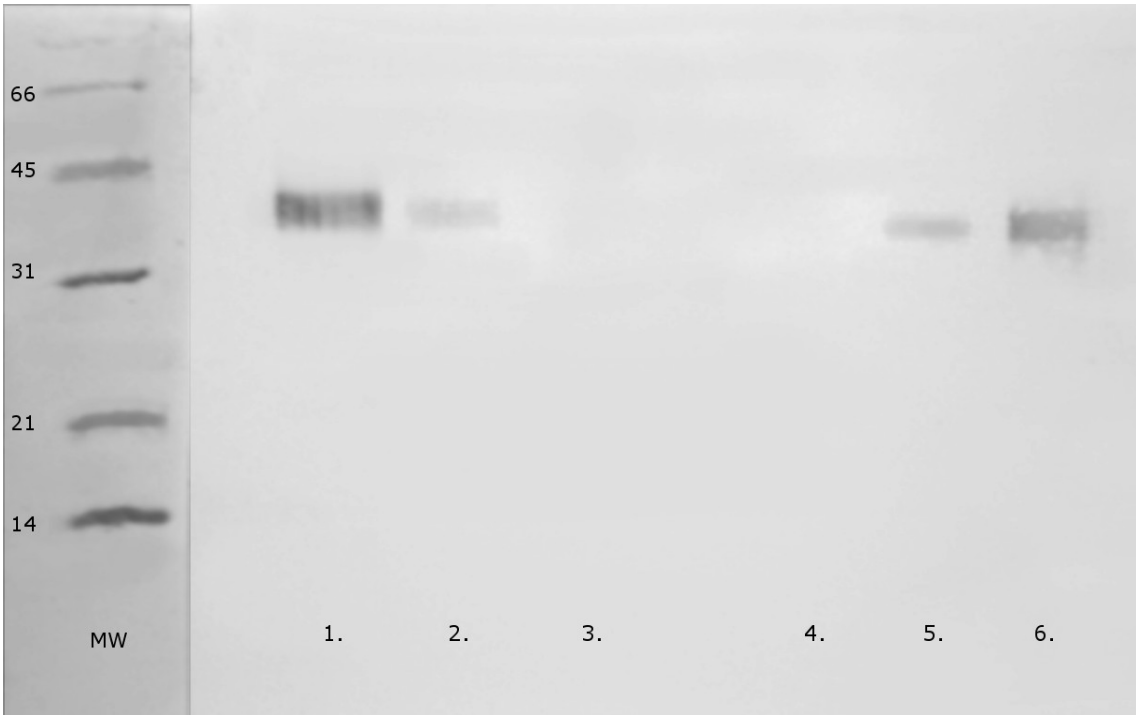
WESTERN BLOT PROTOCOL

Urinary Trypsin Inhibitor Human, Rabbit Polyclonal Antibody

Cat. No.: RD181343100

Western Blot staining of a control protein



MW Marker.: MW: 97, 66, 45, 31, 21, 14 kDa, (Bio-Rad, USA),
Band of 97 kDa is not showen

Lane 1: Urinary Trypsin Inhibitor 100 ng/Lane, Reducing
Lane 2: Urinary Trypsin Inhibitor 10 ng/Lane, Reducing
Lane 3: Urinary Trypsin Inhibitor 1 ng/Lane, Reducing
Lane 4: Urinary Trypsin Inhibitor 1 ng/Lane, Non-reducing
Lane 5: Urinary Trypsin Inhibitor 10 ng/Lane, Non-reducing
Lane 6: Urinary Trypsin Inhibitor 100 ng/Lane, Non-reducing

Human protein Urinary Trypsin Inhibitor (isolated from human urine) was subjected to SDS PAGE followed by Western Blot with Urinary Trypsin Inhibitor Human, Rabbit Polyconal Antibody (RD181343100) at a concentration of 1 µg/ml. Stained with DAB.

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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:
14% 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 15 min and than at 200 V for 45 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 15 V 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 120 min at RT.

Blocking buffer:
0.05 M Tris, 0.15 M NaCl, 0.05% Tween, 0.05% Gelatine, 0.02% Thimerosal

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3. DETECTION:

Detection of a protein Urinary Trypsin Inhibitor Human (isolated from human urine)

Primary antibody:
Urinary Trypsin Inhibitor Human, Rabbit Polyconal Antibody (RD181343100) - 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