

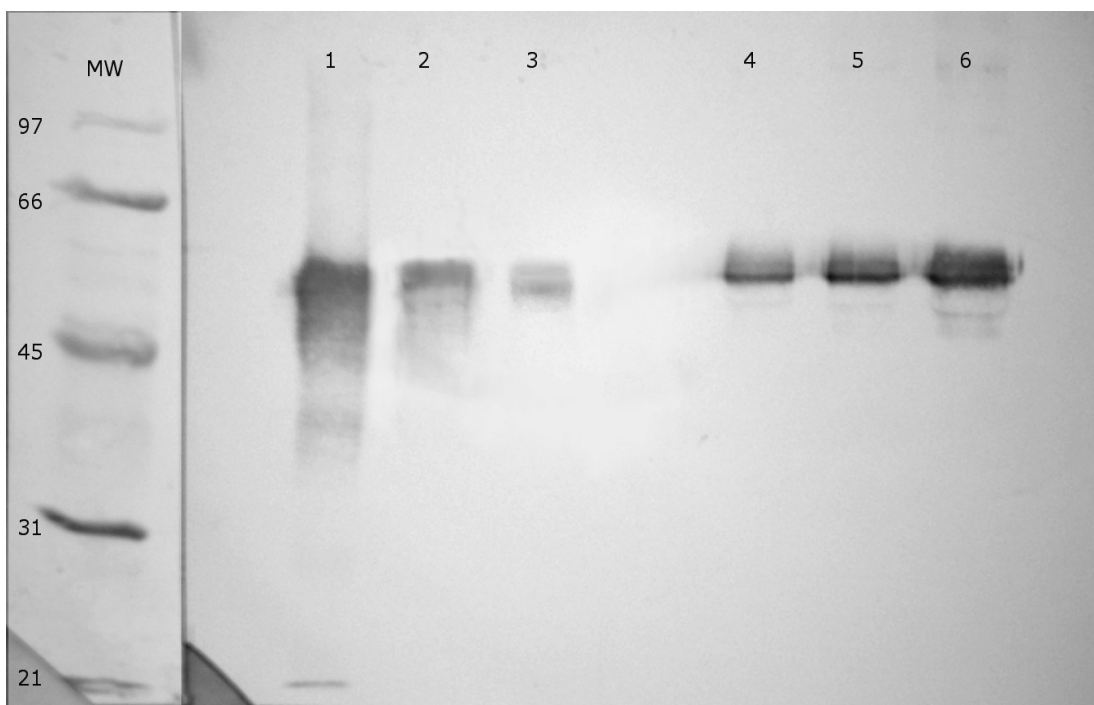
## WESTERN BLOT PROTOCOL

### Fetuin-A (AHSG) Human, Rabbit Polyclonal Antibody

Cat. No.: RD181036100

#### Fig.1a: Western Blot staining of Human Fetuin-A (AHSG)

SDS-PAGE separation under non-reducing conditions

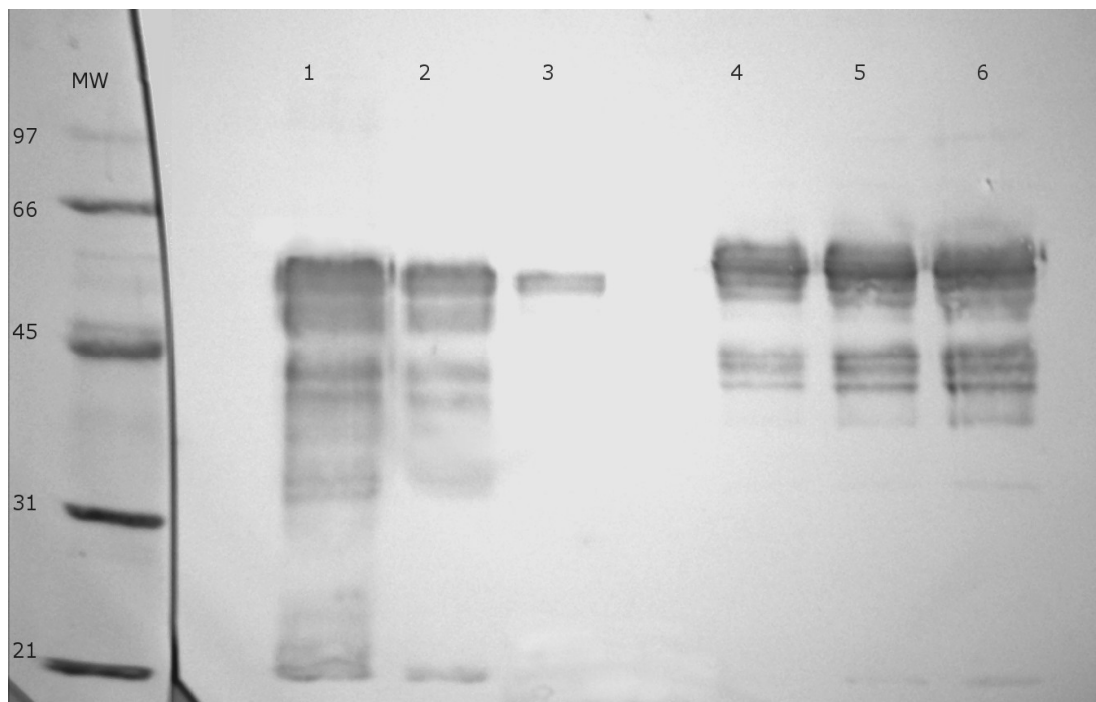


**MW Marker.:** MW: 97, 66, 45, 31, 21 kDa, (Bio-Rad, USA),

- Lane 1:** Fetuin-A Human, Native 100 ng/Lane, Non-reducing
- Lane 2:** Fetuin-A Human, Native 10 ng/Lane, Non-reducing
- Lane 3:** Fetuin-A Human, Native 1 ng/Lane, Non-reducing
- Lane 4:** Human Serum 600x in sample buffer, Non-reducing
- Lane 5:** Human Serum 400x in sample buffer, Non-reducing
- Lane 6:** Human Serum 200x in sample buffer, Non-reducing

**Fig.1b: Western Blot staining of Human Fetuin-A (AHSG)**

SDS-PAGE separation under reducing conditions

**MW Marker.:** MW: 97, 66, 45, 31, 21 kDa, (Bio-Rad, USA),**Lane 1:** Fetuin-A Human, Native 100 ng/Lane, Reducing**Lane 2:** Fetuin-A Human, Native 10 ng/Lane, Reducing**Lane 3:** Fetuin-A Human, Native 1 ng/Lane, Reducing**Lane 4:** Human Serum 600x in sample buffer, Reducing**Lane 5:** Human Serum 400x in sample buffer, Reducing**Lane 6:** Human Serum 200x in sample buffer, Reducing

Fetuin-A (AHSG) NATIVE, Human Plasma (RD172037100) and diluted Human Serum sample were subjected to SDS PAGE under non-reducing conditions (Fig.1a) and under reducing conditions (Fig.1b). Separated proteins were detected in Western Blot with Fetuin-A (AHSG) Human, Rabbit Polyclonal Antibody (RD181036100) diluted at a concentration of 0.75 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:

10% 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 transferred protein was blocked in a blocking buffer for 2 hours 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 Fetuin-A (AHSG) NATIVE, Human Plasma (RD172037100)** (BioVendor – Laboratorní medicína s.r.o., Czech Republic)

Primary antibody:

**Fetuin-A (AHSG) Human, Rabbit Polyclonal Antibody (RD181036100)** - concentration 0.75 µg/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