

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects human ECM-1 in direct ELISAs and Western blots. In direct ELISAs, less than 10% cross-reactivity with recombinant mouse ECM-1 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human ECM-1 Ala20-Glu540 Accession # AAH23505
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 µg/mL	See Below

DATA

Western Blot

Detection of Human ECM-1 by Western Blot. Western blot shows lysates of COLO 205 human colorectal adenocarcinoma cell line and SK-Mel-28 human malignant melanoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human ECM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3937) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ECM-1 at approximately 75 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry

ECM-1 in Human Colon Cancer Tissue. ECM-1 was detected in immersion fixed paraffin-embedded sections of human colon cancer tissue using 15 µg/mL Sheep Anti-Human ECM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3937) overnight at 4 °C. Tissue was stained with the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific labeling was localized to the plasma membrane of epithelial cells. Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

Simple Western

Detection of Human ECM-1 by Simple Western™. Simple Western lane view shows lysates of COLO 205 human colorectal adenocarcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for ECM-1 at approximately 90 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human ECM-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3937) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Extracellular matrix protein-1 (ECM-1) is an 85 kDa, secreted glycoprotein important in connective tissue organization (1-3). Of three identified splice variants the 540 amino acid (aa) form, ECM-1a, is the most widely expressed, with the highest expression in the placenta and heart (2). ECM-1b (415 aa) is found only in tonsil and associated with suprabasal keratinocytes (2, 4). Since ECM-1b expression is differentiation-dependent, a role in terminal keratinocyte differentiation has been suggested (4). ECM-1c (559 aa) accounts for approximately 15% of skin ECM-1 (5). Human ECM-1a contains a 19 aa signal peptide and a 521 aa secreted portion that includes an N-terminal proline-rich, cysteine-free region, two tandem repeat domains, and a C-terminal domain. There are six repeats of a CC(X₇₋₁₀)C motif (x = any aa) within the tandem repeat and C-terminal domains. These motifs are involved in ligand binding to members of the albumin family, and are expected to form two (in ECM-1b) or three (in ECM-1a) "double loop" structures (2). Mature human ECM-1a shows 69%, 71%, 72%, and 76% aa identity with corresponding isoforms of mouse, rat, canine, and bovine ECM-1, respectively. ECM-1 is over-expressed in many malignant epithelial tumors and has demonstrated angiogenic activity (6, 7). A variety of ECM-1 mutations, mainly within the first tandem repeat, are considered causative of lipoid proteinosis, a condition showing thickened and irregular extracellular matrix within connective tissue (8). In the autoimmune condition lichen sclerosis, auto-antibodies mainly recognize the second tandem repeat or the C-terminus of ECM-1 (9). These domains also bind the extracellular matrix molecules fibulin-1 and perlecan (5, 10). The phenotypes of lipoid proteinosis and lichen sclerosis support a role for ECM-1 as a "biological glue" in the dermis (1).

References:

1. Chan, I. (2004) *Exp. Dermatol.* **29**:52.
2. Smits, P. *et al.* (1997) *Genomics* **45**:487.
3. Bhalerao, J. *et al.* (1995) *J. Biol. Chem.* **270**:16385.
4. Smits, P. *et al.* (2000) *J. Invest. Dermatol.* **114**:718.
5. Mongiat, M. *et al.* (2003) *J. Biol. Chem.* **278**:17491.
6. Han, Z. *et al.* (2001) *FASEB J.* **15**:988.
7. Wang, L. *et al.* (2003) *Cancer Lett.* **200**:57.
8. Hamada, T. *et al.* (2003) *J. Invest. Dermatol.* **120**:345.
9. Oyama, N. *et al.* (2004) *J. Clin. Invest.* **113**:1550.
10. Fujimoto, N. *et al.* (2005) *Biochem. Biophys. Res. Commun.* **333**:1327.