

HMGB1 Isoforms Kit

Product Number: *****

Expiration date: *****

Kit description:

HMGB1 Isoforms Kit is an explorative kit thought to test different redox isoforms of HMGB1. It contains:

- Fully reduced HMGB1
- Disulfide HMGB1
- Terminally oxidized HMGB1

All the proteins corresponds to the human sequence and are produced in E.coli. The protein is free from LPS (<0.1EU/mL).

Different activities of HMGB1 have been attributed to different biochemical forms that bind to specific receptors (Yang et al, 2012).

- Fully reduced HMGB1 does not induce cytokine/chemokine secretion and is tested in migration assay.
- Disulfide HMGB1 is tested for the ability to stimulate cytokine production in human macrophages.
- Terminally oxidized-HMGB1 has no activity, either as a chemoattractant or in cytokine stimulation.

Reagents format:

All the proteins in the kit have no tags or additional amino acids.

Lyophilized Fully reduced HMGB1 once reconstituted will be dissolved in a solution containing 50 mM HEPES buffer, pH 7.9, 500 mM NaCl and 0.5 mM DTT.

Lyophilized Disulfide HMGB1 and Terminally oxidized HMGB1 once reconstituted will be dissolved in a solution containing 50 mM HEPES buffer, pH 7.9 and 500 mM NaCl.

Kit storage: 2-8°C. Once the proteins are resuspended they can be stored frozen (-20°C).

How to use the proteins:

See the attached datasheets.

This product is for research use only.



Fully reduced-HMGB1, LPS-free

Expiration date: *****

Batch number: *****

Batch concentration: ***** mg/mL after addition of
***** μ L of distilled water.

Product Description:

HMGB1 is a 25 kDa nuclear protein, present in almost all mammalian cells. The protein is virtually identical (213/215 aa) in human, mouse, rat. This product corresponds to the human sequence and is produced in *E.coli*. Fully reduced-HMGB1 (complete notation: HMGB1C23hC45hC106h - Antoine J. *et al* (2014).Mol Med) forms complex with CXCL12 and has chemoattractant activity. It DOES NOT induce cytokine/chemokine secretion when given to target cells. The protein is free from LPS (<0.1EU/mL).

The product contains <0.006% v/v of Triton X-114 due to LPS removal procedure.

Reagent format:

Fully reduced-HMGB1 we provide is the natural protein, with no tags or additional amino acids.

The lyophilized protein once reconstituted will be dissolved in a solution containing 50 mM HEPES pH 7.9, 500 mM NaCl, DTT 0,5 mM.

Storage: 2-8°C. The protein once resuspended can be stored frozen (-20°C).

Oxidation of cysteine 106 makes the protein inactive (Kazama *et al*, Immunity 2008; 29, 21-32).

To avoid cysteine oxidation DTT 0.5 mM is added during protein purification.

How to use product:

The product can be used in cell migration assays, both *in vitro* and *in vivo*; maximum activity is at 1 nM (Palumbo *et al*, 2004). Intraperitoneal injection in the mouse recruits neutrophils, monocytes and macrophages (Penzo *et al*, 2010).

This product is for research use only

References:

- Agnieszka I. *et al* (2021) Antioxidants The Time-Course of Antioxidant Irisin Activity: Role of the Nrf2/HO-1/HMGB1 Axis 10(1):88
- Li R. *et al* (2021) Lipopolysaccharide-Activated Canine Platelets Upregulate High Mobility Group Box-1 via Toll-Like Receptor. Front Vet Sci. 8:674678
- Piao C. *et.al* (2020) An 8-Hydroxy-Quinoline Derivative Protects Against Lipopolysaccharide-Induced Lethality in Endotoxemia by Inhibiting HMGB1-Mediated Caspase-11 Signaling. Frontiers in Pharmacology. 12:673818
- Piao C. *et al* (2020) A RAGE-antagonist peptide potentiates polymeric micelle-mediated intracellular delivery of plasmid DNA for acute lung injury gene therapy. 12(25):13606-13617

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MGKGDPPKPR GKSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKGLG EMWNTAADD KQPYEKKAAC
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Fig. 1. Fully reduced-HMGB1 sequence

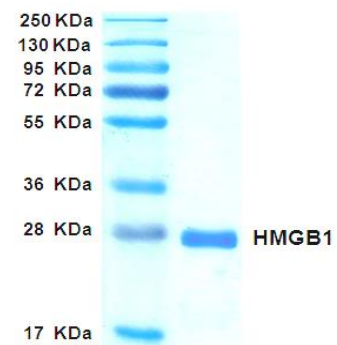


Fig. 2. SDS-PAGE with Coomassie Blue staining

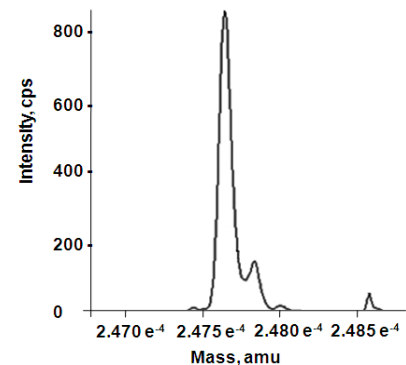


Fig. 3. Reconstructed molecular weight from MS

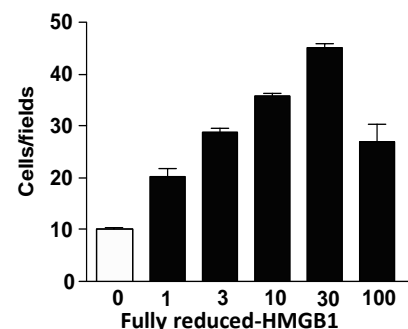


Fig. 4. Migration assay with 3T3 mouse cells



Disulfide-HMGB1, LPS-free

Expiration date: *****
Batch number: *****
Batch concentration: ***** mg/mL after addition of
***** μ L of distilled water.

Product Description:

Disulfide-HMGB1 (complete notation: HMGB1C23-C45C106h - Antoine J. *et al* (2014).Mol Med) can induce cytokine and chemokine production in monocytes and other inflammatory cells. This activity depends on a specific redox state of HMGB1 (Venereau *et al*, 2012).

This product is produced in *E.coli* and is tested for the ability to stimulate cytokine production in mouse macrophages. The protein is free from LPS (<0.1EU/mL). The product contains <0.006% v/v of Triton X-114 due to LPS removal procedure.

Reagent format:

The Disulfide-HMGB1 protein we provide is the natural protein, with no tags or additional amino acids.

The lyophilized protein once reconstituted will be dissolved in a solution containing 50 mM HEPES buffer, pH 7.9 and 500 mM NaCl.

Storage: 2-8°C. The protein once resuspended can be stored frozen (-20°C).

How to use product:

The product can be used as a pro-inflammatory mediator (Venereau *et al*, 2012).

This product is for research use only

References:

- Ge X. *et al* (2024) Redox-sensitive high-mobility group box-1 isoforms contribute to liver fibrosis progression and resolution in mice J.Hepatol PMID: 37989401
- Zhou X. *et al* (2024) Glycyrrhizin Protects Submandibular Gland Against Radiation Damage by Enhancing Antioxidant Defense and Preserving Mitochondrial Homeostasis. Antioxid Redox Signal PMID: 38069572
- Venereau E. *et al* (2012) Mutually exclusive redox forms of HMGB1 promote cell recruitment or proinflammatory cytokine release J Exp Med. 209: 1519–1528.

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MGKGDPPKPR GKSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGKF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKKLG EMWNNNTAADD KQPYEKKAAC
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SKKKKEEEDD EDEEDEDEEEE EEEDEDEDEE
DDDDE
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Fig. 1. Disulfide-HMGB1 sequence

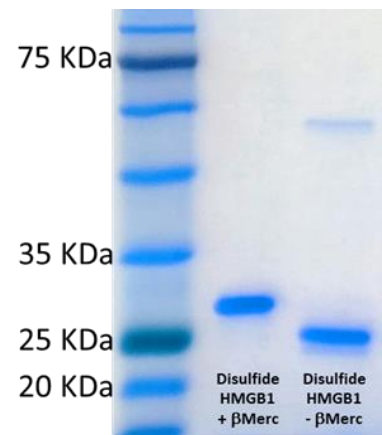


Fig. 2. SDS-PAGE with Coomassie Blue staining

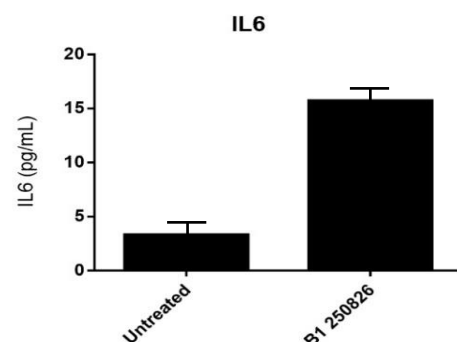
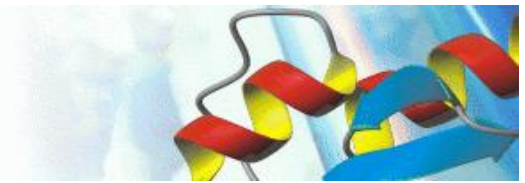


Fig. 3. Disulfide-HMGB1 induces cytokine production. Mouse macrophages were exposed to 10 mg/ml HMGB1 for 24 hours at 37°C, and the levels of IL-6 were measured by ELISA relative to unexposed macrophages.



Terminally oxidized-HMGB1

Expiration date: *****
Batch number: *****
Batch concentration: ***** mg/mL after addition of
***** μ L of distilled water.

Product Description:

HMGB1 is a 25 kDa nuclear protein, present in almost all mammalian cells.

Terminally oxidized-HMGB1 has all the cysteines oxidized to sulfonates and has no activity, either as a chemoattractant or in cytokine stimulation (Kazama et al, 2008 ;Yang et al, 2012).

This product is produced in *E.coli*. The protein is free from LPS (<0.1EU/mL). The product contains <0.006% v/v of Triton X-114 due to LPS removal procedure.

Reagent format:

The terminally oxidized-HMGB1 protein we provide is the natural protein, with no tags or additional amino acids.

The lyophilized protein once reconstituted will be dissolved in a solution containing 50 mM HEPES pH 7.9, 500 mM NaCl.

Storage: 2-8°C. The protein once resuspended can be stored frozen (-20°C).

This product is for research use only

References:

- Ye Y. *et al* (2019) The Role of High Mobility Group Box 1 in Ischemic Stroke. *Front Cell Neurosci* 2:13:127
- Venereau E. *et al*. (2013) HMGB1 and leukocyte migration during trauma and sterile inflammation. *Mol Immunol.* 55(1):76-82
- Yang X. *et al* (2012) Redox modification of cysteine residues regulates the cytokine activity of HMGB1. *Mol Med.*00389

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MGKGDPPKPR GKSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKKLG EMWNNTAADD KQPYEKKAAC
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SKKKKEEEDD EEDEEDEEEE EEEDEDEEEE
DDDDE
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Fig. 1. Terminally-HMGB1 sequence

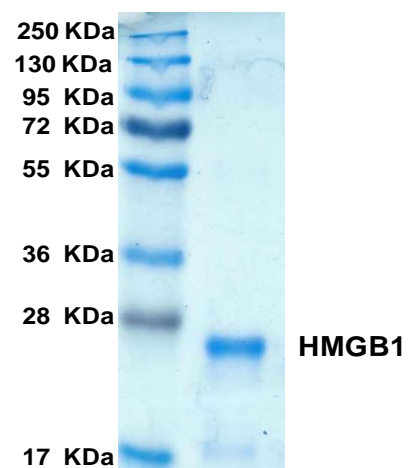


Fig. 2. SDS-PAGE with Coomassie Blue staining