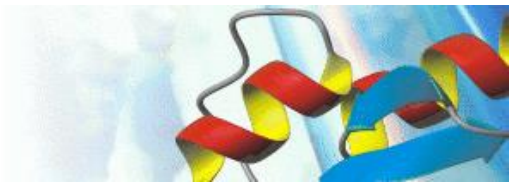


HMGB1 Isoforms Kit

Product Number: HM-030; HM-031
Expiration date: (depends on batch)

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*. They contains only trace amounts of LPS (<0.4 ng/mg protein).
Different activities of HMGB1 have been attributed to different biochemical forms that bind to specific receptors (Yang *et al*, 2012).

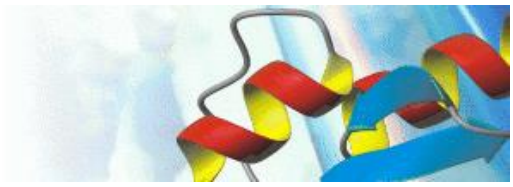
- Fully reduced HMGB1 does not induce cytokine/chemokine secretion and is tested in migration assay.
- Disulfide HMGB1 is tested for the ability to stimulate cytokine production in human macrophages.
- Terminally oxidized-HMGB1 has no activity, either as a chemoattractant or in cytokine stimulation.

Reagents format:
All the proteins in the kit have no tags or additional amino acids.
Fully reduced and Terminally oxidized HMGB1 are lyophilized from 50 mM HEPES buffer, pH 7.9, 500 mM NaCl and 0.5 mM DTT.
Disulfide HMGB1 is lyophilized from 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: (depends on batch)
Batch number: (each batch has a specific tracking number)
Batch concentration: (depends on batch) after addition of (depends on batch) µL of distilled water.

Product Description:
HMGB1 is a 25 kDa nuclear protein, present in almostall mammalian cells. The protein is almost identical (213/215 aa) in human, mouse, rat. This product corresponds to the rat 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 product contains only trace amounts of LPS (<0.4 ng/mg protein) and it is tested for the ability to induce fibroblast migration.

Reagent format:
Fully reduced-HMGB1 we provide is the natural protein, with no tags or additional amino acids.
Fully reduced-HMGB1 is lyophilized from 50 mM HEPES buffer, pH 7.9, 500 mM NaCl and 0.5 mM DTT.

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:
Scaffidi *et al* (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418: 191-195
Palumbo *et al* (2004) Extracellular HMGB1, a signal of tissue damage, induces mesoangioblast migration and proliferation. J Cell Biol 164:441-9
Kazama *et al* (2008) Induction of immunological tolerance by apoptotic cells requires caspase-dependent oxidation of high-mobility group box-1 protein. Immunity 29: 21-32
Schiraldi *et al* (2012) HMGB1 promotes recruitment of inflammatory cells to damaged tissues by forming a complex with CXCL12 and signaling via CXCR4. JEM 209:551-563

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MGKGDPKKPR GKMSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGKF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKKLG EMWNNTAADD KQPYEKKAAK
LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK
SKKKKEEEDD EEDEEDEEEE EEEEDEDEEE
DDDDE
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Fig. 1. Fully reduced-HMGB1 sequence

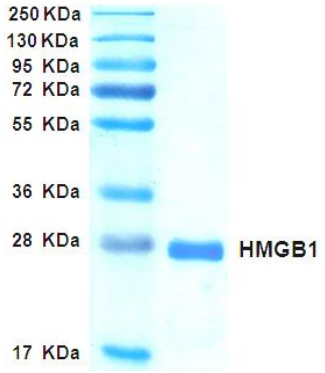


Fig. 2. SDS-PAGE with Coomassie Blue staining

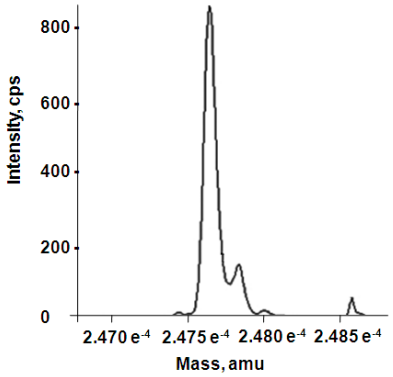


Fig. 3. . Reconstructed molecular weight from MS spectrum.

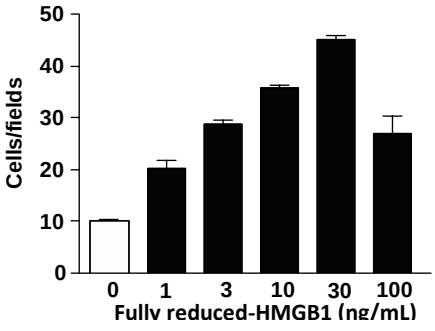
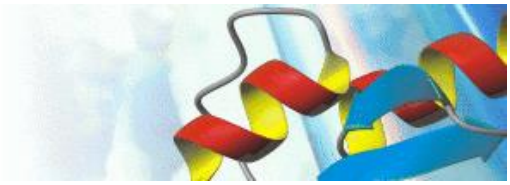


Fig. 4. Migration assay with 3T3 mouse cells



Disulfide-HMGB1, LPS-free

Expiration date: (depends on batch)
Batch number: (each batch has a specific tracking number)
Batch concentration: (depends on batch) after addition of (depends on batch) µL of distilled water.

Product Description:
Disulfide-HMGB1 can induce cytokine and chemokine production in monocytes and other inflammatory cells. This activity depends on a specifc redox state of HMGB1 (Venereau et al,2012).
This product is produced in E.coli. It contains only trace amounts of LPS (<0.4 ng/mg protein), and is tested for the ability to stimulate cytokine production in human macrophages.

Reagent format:
The Disulfide-HMGB1 protein we provide is the natural protein, with no tags or additional amino acids.
Disulfide-HMGB1 is lyophilized from 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 (Yang *et al*, 2012).

This product is for research use only

References:
Wang *et al* (1999) HMG-1 as a late mediator of endotoxin lethality in mice.Science 285:248-51
Andersson *et al* (2000) High mobility group 1 protein (HMG-1) stimulates proinflammatory cytokine synthesis in human monocytes. J Exp Med 192:565-70
Scaffidi *et al* (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418: 191-195
Yang *et al* (2012) Redox modification of cysteine residues regulates the cytokine activity of HMGB1. Mol Med 2011 Nov 7. doi: 10.2119/molmed.2011.00389. [Epub ahead of print] PMID: 22105604

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MGKGDPKKPR GKMSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGKF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKKLG EMWNNTAADD KQPYEKKAAK
LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK
SKKKKEEEDD EEDEEDEEEE EEEEDEDEEE
DDDDE
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Fig. 1. Disulfide-HMGB1 sequence

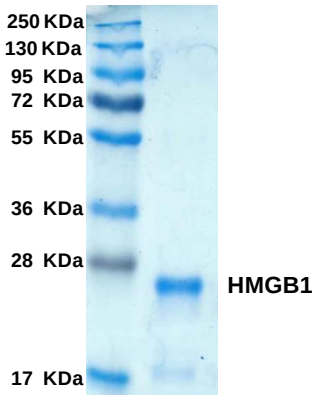


Fig. 2. SDS-PAGE with Coomassie Blue staining

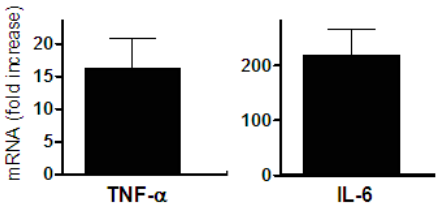
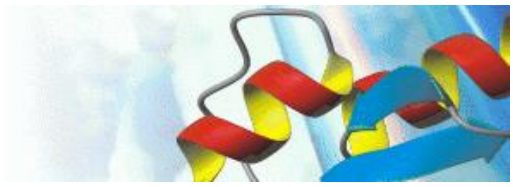


Fig. 3. Disulfide-HMGB1 induces cytokine production. Human monocyte-derived macrophages were exposed to 10 µg/ml HMGB1 for 4 hours at 37°C, and the levels of TNF-α and IL-6 mRNAs were measured by qPCR, relative to unexposed macrophages.



Terminally oxidized HMGB1, LPS-free

Expiration date: (depends on batch)
Batch number: (each batch has a specific tracking number)
Batch concentration: (depends on batch) after addition of (depends on batch) µL 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.
It contains only trace amounts of LPS (<0.4 ng/mg protein).

Reagent format:
The terminally oxidized-HMGB1 protein we provide is the natural protein, with no tags or additional amino acids.
Terminally oxidized-HMGB1 is lyophilized from 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).

This product is for research use only

MGKGDPKKPR GKMSSYAFFV QTCREEHKKK
HPDASVNFSE FSKKCSERWK TMSAKEKGKF
EDMAKADKAR YEREMKTYIP PKGETKKKFK
DPNAPKRPPS AFFLFCSEYR PKIKGEHPGL
SIGDVAKKLG EMWNNTAADD KQPYEKKAAK
LKEKYEKDIA AYRAKGKPDA AKKGVVKAEK
SKKKKEEEDD EEDEEDEEEE EEEEDEDEEE
DDDDE

Fig. 1. HMGB1 sequence

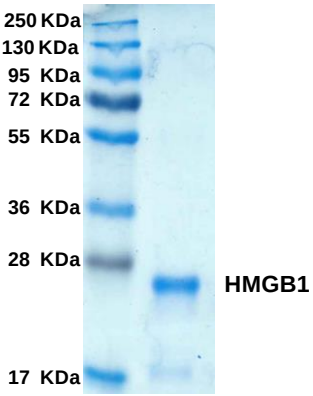


Fig. 2. SDS-PAGE with Coomassie Blue staining

References:
Yang *et al* (2012) Redox modification of cysteine residues regulates the cytokine activity of HMGB1. Mol Med 2011 Nov 7. doi: 10.2119/molmed.2011.00389. [Epub ahead of print] PMID: 22105604
Venereau *et al*. HMGB1 and leukocyte migration during trauma and sterile inflammation. Mol Immunol. 2013 PMID:23207101