



Fully reduced HMGB1, LPS free

Product Number: HM-114;HM-115;HM-116; HM-117

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. The protein is almost 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 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), thawed and re-frozen.

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

Penzo *et al* (2010) Inhibitor of NF- κ B kinases a and b are both essential for HMGB1-mediated chemotaxis. J Immunol 184: 4497-509

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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MGKGDPPKPR  GKMSYYAFFV  QTCREEHKKK
HPDASVNFSE  FSKKCSERWK  TMSAKEKGKF
EDMAKADKAR  YEREMKTYIP  PKGETKKKFK
DPNAPKRPPS  AFFLFCSEYR  PKIKGEHPGL
SIGDVAKGLG  EMWNNNTAAD  KQPYEKKAAC
LKEKYEKIDIA  AYRAKGKPPA  AKKGVVKAEK
SKKKKEEEDD  EEDEEEDDEE  EEEDEDEEEE
DDDDE
    
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Fig. 1. Fully reduced HMGB1 sequence

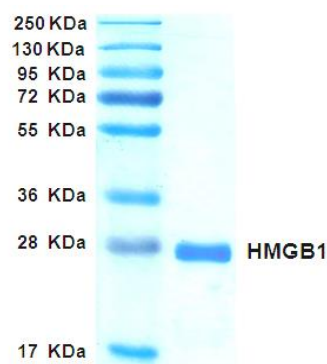


Fig. 2. SDS-PAGE with Coomassie Blue staining

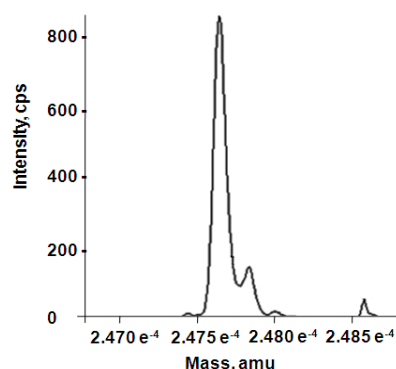


Fig. 3. Reconstructed molecular weight from MS

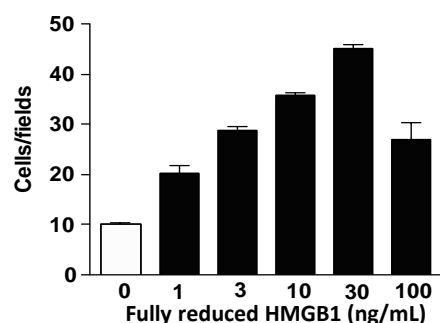


Fig. 4. Migration assay with 3T3 mouse cells