



# HMGB-1 (Rat) ELISA Kit

08/20

(Catalog # E4865-100; 96 assays, Storage at 4°C)

## I. Introduction:

High-mobility group protein B1 (HMGB1), also known as high-mobility group protein 1 (HMG-1) and amphoterin, is a member of the HMGB family consisting of three members, HMGB1, HMGB2 and HMGB3. HMGB1 is a non-histone architectural chromosomal protein ubiquitously present in all vertebrate nuclei and binds double-stranded DNA without sequence specificity. It is also involved in inflammation and damage by binding to TLR4, which mediates HMGB1-dependent activation of macrophage cytokine release. This positions HMGB1 at the intersection of sterile and infectious inflammatory responses. HMGB1 has been studied as a DNA vaccine adjuvant and a target for cancer therapy. BioVision's HMGB-1 (Rat) ELISA Kit is based on the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to rat HMGB1. Standards or samples are added to the micro ELISA plate wells that bind to the specific antibody. Then a biotinylated detection antibody specific for rat HMGB1 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. The wells are washed, a TMB substrate solution is added to the wells and blue color develops in proportion to the amount of HMGB-1 bound. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of rat HMGB1. The concentration of rat HMGB1 in the samples can be calculated by comparing the OD of the samples to the standard curve.

## II. Applications:

in vitro quantitative determination of rat HMGB-1 concentrations in serum, plasma and other biological fluids.

**Sensitivity:** 18.75 pg/mL

**Detection Range:** 31.25-2000 pg/mL

**Specificity:** No significant crossreactivity or interference between rat HMGB-1 and analogues was observed.

**Precision:** Coefficient of variation is < 10%.

## III. Sample Type:

Serum, plasma and other biological fluids

## IV. Kit Contents:

Components	E4865-100	Part Number	Storage
Micro ELISA Plate	8 wells x12 strips	E4865-100-1	-20°C
Reference Standard	2 vials	E4865-100-2	-20°C
Biotinylated Detection Ab (100x)	120 µl	E4865-100-3	-20°C
HRP Conjugate (100x)	120 µl	E4865-100-4	-20°C (protect from light)
Reference Standard & Sample Diluent	20 ml	E4865-100-5	4°C
Biotinylated Detection Antibody Diluent	14 ml	E4865-100-6	4°C
HRP Conjugate Diluent	14 ml	E4865-100-7	4°C
Wash Buffer (25X)	30 ml	E4865-100-8	4°C
Substrate Reagent	10 ml	E4865-100-9	4°C (protect from light)
Stop Solution	10 ml	E4865-100-10	4°C
Plate Sealer	4	E4865-100-11	4°C

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Clean Eppendorf tubes for preparing standards or sample dilutions

## VI. Storage and Handling:

An unopened kit can be stored at 2-8°C for 1 month. If the kit is not used within 1 month, store the items separately according to the above mentioned conditions once the kit is received.

## VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (25X):** Dilute 30 ml of Concentrated Wash Buffer with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer. (Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved)
- **Biotinylated Detection Antibody working solution:** Calculate the required amount (100 µL/well). Centrifuge the stock tube before use; dilute the 100x Concentrated Biotinylated Detection Antibody to 1x working solution with Biotinylated Detection Antibody Diluent.



