

# Human High Mobility Group Protein B1 (HMGB1) ELISA Development Kit

Catalogue No.:BTA14625

High Mobility Group Protein B1 (HMGB1) ELISA Development Kit for use in Sandwich ELISA assay development.

This ELISA Development Kit contains:

Component	5 × 96 tests	15 × 96 tests
Pretreated 96-well ELISA Plate	5	15
Capture Antibody	120 µl	350 µl
Biotin-Conjugated Detection Antibody	120 µl	350 µl
HRP-Conjugate	120 µl	350 µl
Standard	1 vial	3 vials

Please note that quantities and concentrations may change between different batches.

It is recommended to use this ELISA Development Kit with [abx471002 ELISA Development Support Kit \(Sandwich Method\)](#).

**Target:** High Mobility Group Protein B1 (HMGB1)

**Reactivity:** Human

**Tested Applications:** ELISA

**Recommended dilutions:** Capture Antibody: 1/500 - 1/1000, Biotin-conjugated Detection Antibody: 1/500 - 1/1000, HRP-Conjugate: 1/500 - 1/1000. Optimal dilutions/concentrations should be determined by the end user.

**Reconstitution:** Reconstitute the standard with 1 ml of Standard Diluent, then serially dilute as required.

**Storage:** Aliquot and store at -20°C in the dark. Avoid repeated freeze/thaw cycles.

**UniProt Primary AC:** P09429 ([UniProt](#), [ExpASy](#))

**Test Range:** 31.2 pg/ml - 2000 pg/ml

**Detection Method:** Colormetric

**Assay Type:** Sandwich

**Sample Type:** Serum and plasma.

**Note:** This product is for research use only.

**Directions for use:** Bring all components to room temperature (18-25°C) and briefly spin or centrifuge the vials before use. Working solutions should be prepared and used immediately.

1. Dilute the Capture Antibody to working concentration using Coating Buffer. Immediately coat the 96-well plate with diluted Capture Antibody (100 µl per well). Seal the plate and incubate at 2-8 °C overnight.
2. Remove the liquid from each well. Do not wash. Block the plate with Blocking Buffer (200 µl per well) at 37 °C for 1 hour.
3. Remove the liquid from each well. Do not wash. Either proceed with the following steps immediately or dry the plate at 37 °C for 30 minutes, then store at -20 °C with dessicant for up to 6 months.
4. Add 100 µl of standards or sample into the appropriate wells. Cover with a plate sealer and incubate at 37 °C for 1.5 hours.
5. Remove the liquid from each well. Do not wash. Add appropriately diluted Biotin-Conjugated Detection Antibody (100 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 1 hour.
6. Remove the liquid from each well. Wash with Wash Buffer (350 µl per well) and allow to soak for 1-2 min. Remove the liquid by inverting and tapping the plate on to absorbent paper. Repeat the wash process 3 times.
7. Add appropriately diluted HRP-Conjugate (100 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 30 min.
8. Repeat the wash process in Step 6, for a total of 5 times.
9. Add Substrate Solution (90 µl per well). Cover the plate with a new plate sealer and incubate at 37 °C for 15-30 min. Keep the plate in the dark and avoid exposure to light.
10. Add Stop Solution (50 µl per well). Tap the side of the plate to ensure thorough mixing.
11. Measure the absorbance immediately using a microplate reader set at 450 nm.