

## Human Osteopontin (SPP1) ELISA Development Kit

Catalogue No.:BTA14647

Osteopontin (SPP1) 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).

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:	P10451 ( <u>UniProt</u> , <u>ExPAS</u> ) y
Test Range:	0.312 ng/ml - 20 ng/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. <u>Recommended Procedure:</u>

- 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.
- 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.
- 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.
- 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.
- 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.
- 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.