OHAUS®

Sandwich Enzyme-Linked Immunosorbent Assay

Developed by OHAUS Corporation

Standard Preparation

- 100 µl peptide in coating buffer is added to individual wells of a microtiter plate. Incubate the plate for 2 hours at 37 °C or overnight at 4 °C using OHAUS 2 Block Dry Block Heaters (30392101).
- Remove the coating solution and wash the plate three times by filling the wells with 100 µl PBS-0.05% Tween 20.
- Block the remaining protein-binding sites in the coated wells by adding 100 µl blocking buffer. Incubate for 1 hour at RT with gentle shaking using the OHAUS Incubating Light Duty Orbital Shaker (30391931).
- Wash the plate three times with 100 µl PBS-0.05% Tween 20.
- Add 50 µl of diluted antibody to each well. Incubate the plate at 37° for an hour with gentle shaking.
- Wash the plate six times with 100 µl PBS-0.05% Tween 20.
- Add 50 µl of conjugated secondary antibody, diluted at the optimal concentration in blocking buffer immediately before use. Incubate at 37° for an hour in the OHAUS Incubating Light Duty Orbital Shaker (30391931).
- Wash the plate six times with 100ul PBS-0.05% Tween 20.
- Prepare the substrate reagents at designated concentrations.
- Dispense 50 µl of the substrate solution per well with a multichannel pipette. Incubate the plate at 37° in the dark for 15–30 minutes with the OHAUS Incubating Light Duty Orbital Shaker (30391931) with opaque lid.
- After sufficient color development, add 100 µl of stop solution to the wells.
- Read the absorbance at specified wavelength in a microplate reader.

OHAUS Products Used Within This Procedure

2 Block Dry Block Heaters

Incubating Light Duty Orbital Shaker