5.6.2024

Example of ELISA procedure for KaiSA96-Lockwell plates

Materials and Reagents Preparation

Before starting ELISA, please prepare all the reagents and materials required in the experiment.

Streptavidin Coated Microplate: (Uniogen Cat. No. 41-01) KaiSA96-Lockwell

Wash Buffer: (Uniogen Cat. No. 42-01) Wash Concentrate 25x

Assay Buffer: (Uniogen Cat. No. 42-02) Buffer Solution RED

Conjugate Dilution Buffer: Commercially available ready-to-use HRP-conjugate dilution buffer

TMB Substrate Solution: Commercially available ready-to-use TMB substrate solution

Stop Solution: 0,5 M sulfuric acid (aqueous)

Biotinylated protein/antibody: Depending on the assay, provided by user.

HRP-labelled antibody: Depending on the assay, provided by user.

Samples/Calibrators/Controls: Depending on the assay, provided by user.

Distilled or deionised water

Pipettes and tips

Tubes and vials for making the dilutions

Microplate shaker

Microplate washer: ELISA microplate washer for different formats or for solid microplates

UV/Vis microplate spectrophotometer: Absorbance 450 nm, correction wavelength set to 630 nm

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Recommended protocol

Please note that this protocol is an example of how to perform an ELISA assay using our KaiSA96-Lockwell microplate. To achieve the best performance, further optimization work may be required, depending on the antibodies and analytes used.

1. **Preparation**

   Reconstitute and store all reagents as recommended. Dilute wash buffer (24 parts of deionized water and one part of 25x wash concentrate).

2. **Add biotinylated protein or antibody**

   1) Dilute biotinylated protein or antibody to a concentration you want (usually 1-10 µg/ml) with Assay Buffer to make a working solution.
   2) Add 100 µl of diluted biotinylated molecule to each well and incubate at room temperature for minimum 30 minutes in slow shaking.

3. **Washing**

   Remove the remaining solution by aspiration and wash the wells two times with a microplate washer.

4. **Add samples/calibrators and controls**

   1) Make appropriate dilutions of samples, calibrators and controls into Assay Buffer.
   2) Add 100 µl of diluted samples/calibrators/controls to each well, incubate at room temperature for minimum 45 minutes in slow shaking.

5. **Washing**

   Repeat step 3.

6. **Add HRP-labelled antibody**

   1) Dilute HRP-labelled antibody solution to an appropriate concentration with Conjugate Dilution Buffer.
   2) For all wells, add 100 µl of diluted HRP-labelled antibody solution, and incubate at room temperature for minimum 45 minutes in slow shaking

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7. **Washing**

   Remove the remaining solution by aspiration and wash the wells six times with a microplate washer.

8. **TMB Substrate reaction**

   Add 150 µl of TMB Substrate solution to each well and incubate the microtiter plate for 10 minutes at room temperature, no shaking. Cover the microtiter plate with aluminium foil to protect from light or incubate in a dark place.

9. **Termination**

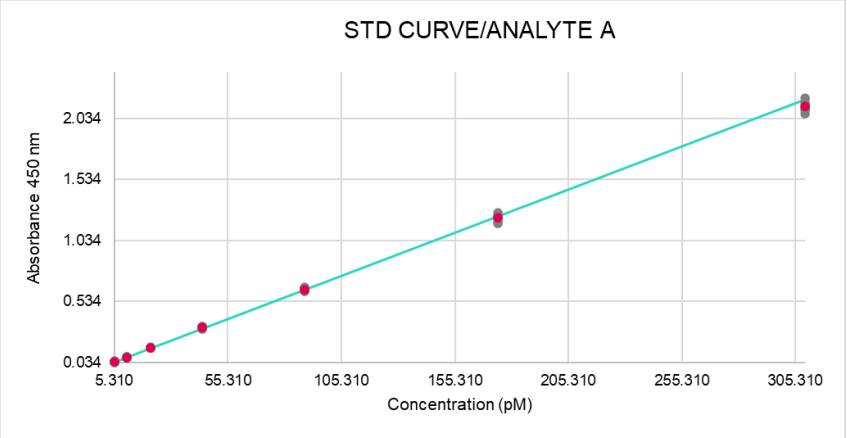
   Add 50 µl of Stop Solution to each well and mix the plate in a plate shaker for 1 minute.

10. **Data Recording**

    Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer within 20 minutes after the addition of Stop Solution.

Example of ELISA std curve

An example standard curve for analyte A using Uniogen KaiSA96-Lockwell plates, analyte specific biotinylated capture antibodies and HRP-conjugated tracer antibodies. Protocol was performed according to steps 1.-10. above.



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