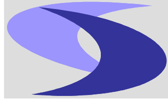


Signosis
Innovative Plate Assay Solutions

UltraSensi DNA-Linked Cytokine Immunoassays

Catalog Number AO-XXXX **(For Research Use Only)**

Introduction

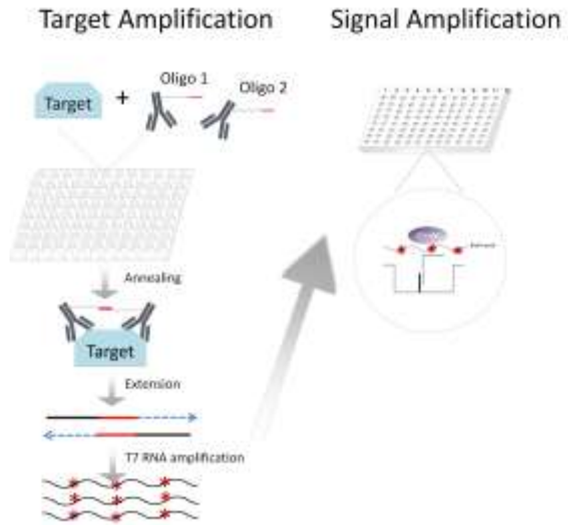
Typical protein detection methods like enzyme-linked immunosorbent assays (ELISAs) are still not sensitive enough to detect low concentrations of important biological markers such as troponin, prostate-specific antigen and some of cytokines, even with improved substrates, e.g. photometric, fluorescence and chemiluminescence-generating compounds. More recent DNA-linked methods for sensitive protein detection have been reported, for example, Immuno-PCR was developed with combining the specificity of antibodies and the amplification power of PCR allowing a 100-fold increase in sensitivity. However, the immuno-PCR hasn't been widely utilized in research commercially due to high background and low signal/noise ratio resulting from exponential amplification, which may affect the sensitivity tremendously.

Now with this technology, Signosis has launched a series UltraSensi DNA-Linker Cytokine Immunoassay kits. The assay can detect the protein level as least as 0.001 pmol with 0.1-1ul of sample amount, particularly well-suited for minuscule amounts of sample material, e.g. few µl of biological material from small animals and from cerebrospinal fluid (CSF), which are often impossible to be identified with current ELISA methods. It has been reported that IL-1b, G-CSF, and b-NGF can't be detected in most of the samples tested due to low circulating levels under normal physiological conditions (<1.5 pg/ml).

Principle of the assay

Signosis is introducing an UltraSensi DNA-Linked Immunoassay Product Line which applied with a close proximity ligation technology. Two DNA oligos conjugated with a pair of target-specific antibodies respectively can be brought closely enough for ligation when the antibodies bind to their target protein simultaneously. The ligated DNA is then extended and is subject to T7 RNA Polymerase linear amplification. The amplified RNA is detected by the following coupled streptavidin-HRP reaction in a rapid plate hybridization assay.

Procedure Overview



Materials provided with the kit

Component	Qty	Store at
96-Well Plate (with aluminum adhesive seal)	1	RT
2X Annealing Buffer	1100µL	-20°C
Antibody-oligo conjugate A	22µL	-20°C
Antibody-oligo conjugate B	22µL	-20°C
Diluent Buffer	100µL	4°C
TF Plate Hybridization Buffer	10mL	RT
5X Plate Hybridization Wash Buffer	15mL	RT
5X Detection Wash Buffer	15mL	RT
Blocking Buffer	30mL	RT
Protein Standards	8µL	-20°C
Primers (F/R mix)	100µL	-20°C
Substrate A	1mL	4°C
Substrate B	1mL	4°C
Streptavidin-HRP Conjugate	20µL	4°C
Substrate Dilution Buffer	8mL	4°C
2X Labeling mix (Pol mixed)	1mL	-20°C

Before Starting the Experiment Prepare the Following:

1. Dilute *2X Annealing buffer* to 1X with ddH2O before use.
2. Warm up *TF Plate Hybridization Buffer* and *Hybridization Wash Buffer* **42°C** before use.
3. Dilute *5X Plate Hybridization Wash Buffer* to 1X with ddH2O before use.
4. Dilute *5X Detection Wash Buffer* to 1X with ddH2O before use.
5. Dilute *Streptavidin-HRP* with Blocking Buffer (1:500 dilution) upon use.

Material required but not provided

- Plate reader for luminescent detection
- PCR machine and PCR tubes
- Hybridization incubator at 42°C
- Plate-Shaker
- Deionized or distilled water.

Please Read the Assay Procedure Before You Begin

Assay procedure

1. Preparation of antibody-oligo conjugates dilutions. For each A and B, dilute 100 times with 1X Annealing buffer in a same tube.
2. Preparation of Standards dilutions (standard curve). See instruction below for standard preparation.

36ul 1X Annealing buffer + 4ul standard ⤼ 20ul
20ul 1X Annealing buffer
20ul 1X Annealing buffer ⤼ 20ul
20ul 1X Annealing buffer ⤼ 20ul
20ul 1X Annealing buffer ⤼ 20ul
20ul 1X Annealing buffer ⤼ 20ul
20ul 1X Annealing buffer ⤼ 20ul
20ul 1X Annealing buffer- control

Annealing

3. Mix the following components for annealing reaction in each PCR tube:

1X Annealing buffer with conjugates A/B	19ul
Standard/Sample	1ul
	20ul

4. Incubate the reaction mix at 37°C for 40 minutes.

Extension

5. Add 1ul of primers (F/R mix) to reaction tube.
6. Perform total 5 cycles of extension reaction.
   1- 94°C 30sec
   2- 54°C 30sec
   3- 72°C 30sec
   4- Repeat above step 1-3, 4 times (total 5 cycles)

T7 RNA Amplification

7. Preparation of T7 RNA amplification reactions.

Extension Reaction	10ul
2X Labeling mix	10ul
	20ul

8. Incubate the reaction mix at 37°C for 1hour.
9. Your reaction is ready to be hybridized on the plate.

Detection

Hybridization of oligos on the Hybridization Plate

10. Add 100ul of warmed Hybridization buffer to each well.
11. Add 5ul of reaction to each well and mix well.
12. Seal the wells with foil film securely and hybridize at 42°C 1.5 hours. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well.

Plate Detection

13. Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
14. Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1X Plate Hybridization Wash Buffer to each well.
15. Completely remove the liquid from the wells by firmly tapping the plate against clean paper towels.
16. Add 200ul of Blocking Buffer to each well and incubate for 5 minutes at room temperature with gentle shaking.
17. Invert the plate over an appropriate container to remove the Blocking Buffer.
18. Dilute Streptavidin-HRP conjugate 500 times with Blocking Buffer and dispense 95ul in each well. Incubate for 20 minutes at room temperature with gentle shaking.
19. Wash the plate 3 times by adding 200ul of 1X Detection Wash Buffer to each well and incubate the plate for 5 minutes with gentle shaking at room temperature.
20. Completely remove the liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, leave the plate inverted on a clean paper towel for 1-2 minutes to remove any excessive liquid.
21. The substrate solution should be prepared fresh:

    Substrate A: Substrate B: Substrate dilution buffer
    1 : 1 : 8
    (for example, 50ul A + 50ul B + 400ul buffer)

22. Add 95ul substrate solution to each well and incubate for 1 minutes.
23. Place the plate in the luminometer. Allow plate to sit inside the machine for 5 minutes before reading. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-10 minutes.