Human anti-LLO IgG Immunoassay Product Number # AKE0007

LLO PROTEIN ELISA KIT
Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-listeriolysin O (LLO) IgG in human serum and plasma in case of Listeria monocytogenes infection.

FOR RESEARCH USE ONLY

NAME AND INTENDED USE

Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-LLO IgG in human serum and plasma to diagnose listerial infection and to screen people for L. monocytogenes exposure.

INTRODUCTION

Listeria monocytogenes is a facultative intracellular Gram-positive food-borne bacterium, increasingly recognized as being responsible for severe infections in both humans and animals (1). Ingestion of contaminated food causes an infection, named listeriosis, which affects especially immunocompromised patients, new-borns and pregnant women and is characterized by a variety of severe syndromes, such as encephalitis, meningoencephalitis, septicemia and abortion (2). Listeriolysin O (LLO), a major virulence factor produced by all pathogenic strains of L. monocytogenes, has been identified as a candidate antigen for a serological assay (3, 4). Antibodies to LLO have been already detected in the serum of goats (5), sheep (6, 7), lambs (4), cows (8) and humans (9) by Western blot, dot blot or ELISA analysis. Detection of anti-LLO antibodies in humans has been proved to be particularly useful for listeriosis diagnosis especially when bacteria cannot be isolated from clinical specimens, owing to the intermittent presence in blood or the inaccessible foci of bacterial replication (10).
L. monocytogenes releases in the culture medium low levels of LLO, therefore the isolation of this toxin requires high culture volumes, is time-consuming and provides a very low yield (11, 12). For this reason the LLO protein, used in the assay as test antigen, was expressed in Escherichia coli and purified as described in (13).

PRINCIPLE OF THE ASSAY

Microtiter strips coated with antigen (listeriolysin O) are incubated with collected samples. During this incubation step, anti-LLO antibodies are bound to antigen forming specific complexes. Antibody excess is removed by washing and in each well the antigen-antibody complex is detected by adding anti-IgG HRP-conjugated globulin. Revelation step is performed incubating the strips with 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as chromogen and reading the absorbance values by ELISA microwell plate reader.

LIMITATIONS OF THE PROCEDURE

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- No drugs have been investigated for assay interference.
- The kit should not be used beyoind the expiration date on the kit label.
- The assay may cross reacts with Clostridium perfringens, Staphylococcus aureus and Streptococcus pyogenes.
- Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature, or kit age can cause variation in binding.

REAGENTS

Microtiter Strips (12 x 8 well strips) coated with LLO protein, blocked with BSA 2% and protected with gelatin.
Plate cover: 1 adhesive plate sealers.
Assay Diluent (Buffer A): 50 ml ready to use, with preservative.
Wash Buffer concentrate (Buffer B): 100 ml 10x to dilute to 1000 ml final volume with distilled water, with preservative.
Conjugated Antibody: 30 µl goat anti-human IgG-HRP conjugated.
Chromogen Solution: 30 ml Buffer C, with preservative; 30 µl buffer C_1; 2 ABTS tablets.

STORAGE

Maintain the kit at 2-4°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm.
- Precision pipettes and pipette tips.
- Glass or plastic pipettes.
- Deionized or distilled water.
- Multi-channel pipette, semi-automated or automated microplate washer.
- 1000 ml graduated cylinder for preparation of Wash Buffer.
- Vortex mixer.
- Glass tubes.

PRECAUTIONS

- The buffer C_1 provided with this kit is H_2O_2 solution 30% m/m (110 volumes). Causes burns, after contact with skin, wash immediately with plenty of water. Wear suitable protective clothing and eye/face protection.
- Do not interchange components between different kits.
- When using the kit check that the reagent solutions are clear.
- Avoid cross-contamination between serum specimens.
- Do not use the kit after the expiration date.
- Treat all specimens and kit serum-based reagents as potentially infectious.

SAMPLE COLLECTION AND STORAGE

Serum – Collect sample in Serum-Use pyrogen/endotoxin free collecting tubes. After blood clotting centrifuge it at approximately 1000 x *g* for 10 min and remove serum from the red cells.
Plasma - Collect blood in Serum-Use pyrogen/endotoxin free collecting tubes with heparin or EDTA and centrifuge it at 1000 x *g* for 10 min. Remove plasma rapidly and carefully.
Storage – Samples can be stored at 2–4°C for up 24 hours after collection. For longer periods samples should be stored frozen. Avoid freeze-thaw cycles.
Recommendation - Before assaying thaw completely samples at room temperature. Do not thaw by heating at 37°C or 56°C.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Assay diluent. Ready to use, utilized this buffer for sera or plasma dilutions in a clean glass vials.
Wash Buffer Concentrate. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 ml of Wash Buffer (Buffer B) concentrate to 1000 ml with distilled water.
Conjugated antibody. Prepare immediately before use. Dilute 1:1000 with buffer A, in a clean glass vial, according to the number of wells to be used (100 µl/well). Do not keep this dilution for further experiments.

Colour solution. Reconstitute immediately before use. Dissolve one ABTS tablet in 12.5 ml of buffer C and mix with 12.5 µl of buffer C1. The Colour solution is light sensitive, avoid prolonged exposure to light.

RUNNING PARTIAL PLATES

This ELISA provides the flexibility to run two partial plates on separated occasions. Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips from the frame and store them at 2-4°C.
Reconstitute the Colour solution as previously described using only one ABTS tablet. Care must be taken to ensure that the remaining buffer C and C_1 are not contamined.

ASSAY METHOD

1. Dilute sera 1:100 in Buffer A.
2. Dispense diluted sera 100 µl/well, in duplicate. Add 100 µl of Buffer A into two wells as reagent blank.
3. Cover the microwells and incubate at 37°C for 60 minutes.
4. Wash the microtiter strips five times with reconstituted Buffer B.
5. Dispense the goat anti-human IgG HRP-conjugated diluted 1:1000 in Buffer A (100 µl/well).
6. Cover the microwells and incubate at 37°C for 60 minutes.
7. Wash the microtiter strips five times with reconstituted Buffer B.
8. Add 100 µl of reconstitute Colour buffer to each well, including reagent blank wells.
9. Cover the microtiter strips and incubate at room temperature for 30 minutes.
10. Read immediately the absorbance at 405 nm using a microplate reader.

ASSAY PROCEDURE SUMMARY

1. Dispense the serum at 1:100 dilution 100 µl /well.
Maintain the plates at 37°C for 60 min.
2. Aspirate and wash five times.
3. Add 100 µl per well of conjugated antibody.
Maintain the plates at 37°C for 60 min.
4. Aspirate and wash five times.
5. Add 100 µl / well of Color Buffer.
Incubate 30 min. RT.
Read at 405 nm.

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