コスモ・バイオ株式会社

diatheva

MOUSE ANTI-TAT Igs ISOTYPING IMMUNOASSAY

Product Number # AKE 0008

FOR RESEARCH USE ONLY

Mouse anti-Tat immunoglobulins isotyping immunoassay

NAME AND INTENDED USE

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of anti-Tat immunoglobulins isotypes in mouse serum and plasma as an aid in the measurement of antibody production in mice immunized with HIV Tat protein.

INTRODUCTION

The HIV-1 regulatory protein Tat is considered an attractive target for the development of a multicomponent vaccine against AIDS. The protein is well conserved among different isolates and thus may be less susceptible to mutation leading to the production of escape virus variants (1-2). Tat is produced early after infection and is essential for virus replication and infectivity (3-4-5). Tat protein is also immunogenic and antibodies against Tat have been found to correlate with delayed disease progression (6-7-8) and may exert protective effects by inhibiting both HIV replication and the effects of extracellular Tat (9-10).
Moreover, Tat is efficiently taken up by monocite-derived dendtritic cells, promotes their maturation and antigen presenting functions (11) directing Th-1 and CTL responses against itself and other Ags since it enters the major histocompatibility complex class I pathway (12). Finally, vaccination of mice with a biologically active Tat protein has been shown to be safe , immunogenic and elicits anti-Tat neutralizing Ab and CTL. In the mouse model, IgG 1 and IgG 3 isotypes are suggestive of a Th2-like immune response whereas the prevalence of IgG 2a isotype suggests of a Th-1 response. . (13) . The presence of IgM and IgA subclasses in mouse serum is also important for evaluating the immunogenicity of Tat as an antigen. Measurement of anti –Tat immunoglobulin isotypes is especially valuable in Tat protein immunized-mouse serum and plasma.

PRINCIPLE OF THE ASSAY

This assay is based on the microplate enzyme linked immunoassay technique.
The antibody against Immunoglobulins isotypes is coated onto the microplate wells. Revelation step includes biotinylated Tat protein, Streptavidin Peroxidase conjugated and ABTS as chromogen.
Immunized mouse serum is pipetted into the wells of microplate precoated with anti-mouse isotypes IgG1, IgG2a, IgG2b, IgG3, IgA, IgM antibodies and pre-blocked. The specific antibody isotype will meet the immunoglobulin sample and form a precipitate that will be revealed by adding biotinylated Tat protein. Biotinylated Tat protein gives the specificity to the mouse anti-Tat immunoglobulins isotyping immunoassay.

LIMITATIONS OF THE PROCEDURE *FOR RESEARCH USE ONLY*

- No drugs have been investigated for assay interference
- The kit should not be used beyond the expiration date labeled on the kit
- Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature, can cause variation in binding efficiency.

REAGENTS

Micro titer Strips: 96 wells polystyrene microplate (12 x 8 wells strips) coated with anti-mouse isotypes IgG1, IgG2a, IgG2b, IgG3, IgA, IgM antibodies and blocked with BSA 1% (2 strips for each immunoglobulin isotype)
Plate Cover: 1 adhesive plate sealers
Assay diluent: (Buffer A) 50 mL ready to use, with preservative
Wash buffer concentrate: (Buffer B) 150 mL 10x to dilute to 1500 mL final volume with distilled water, with preservative
Biotinylated TAT protein: 12 µl of Tat protein biotin conjugated
Chromogen Solution: (Buffer C) 12,5 mL with preservative; (Buffer C_1) 12,5 µl; 1 ABTS tablet.

STORAGE

Maintain the kit at 2-8°C

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm
- Precision pipettes and pipette tips
- Deionized or distilled water
- Multi-channel pipette, squirt bottle, manifold dispenser, or automated microplate washer
- 2000 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.
The antibodies used in this assay as no testing can offer complete assurance of freedom from infectious agents.

SAMPLE COLLECTION AND STORAGE

Serum - Collect samples 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.