HUMAN ANTI-TAT IgG IMMUNOASSAY

Product Number AKE0004

Enzyme linked immunosorbent assay (ELISA) for qualitative and quantitative determination of anti- Tat IgG concentrations in human serum and plasma as an aid in the measurement of antibody production in human immunized with (HIV) Tat protein and in case of and in case of HIV infection.

FOR RESEARCH USE ONLY .

NAME AND INTENDED USE

Enzyme linked immunosorbent assay (ELISA) for the quantitative determination of anti- Tat IgG concentrations in human serum and plasma as an aid in the diagnosis of antibody production in human immunized with (HIV) Tat protein.

INTRODUCTION

The HIV-1 regulatory proteins Tat are considered attractive targets for the development of a multicomponent vaccine against HIV-1 infection. The protein is well conserved among different isolates and thus may be less susceptible to mutation leading to the production of escape virus variants. Tat is produced early after infection and is essential for virus replication and infectivity. Tat protein is also immunogenic and antibodies (Ab) against Tat have been found to correlate with delayed disease progression and may exert protective effects by inhibiting both HIV replication and the effects of extracellular Tat. Moreover Tat is efficiently taken up by monocite-derived dendritic cells, promotes their maturation and antigen presenting functions directing Th-1 and CTL responses against itself and other Ags since it enters the major histocompatibility complex class I pathway. 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.
The Tat protein was expressed in *Escherichia Coli* and purified by heparin sepharose affinity chromatography. The purified Tat protein is >95% pure and fully monomeric, as tested by sodium dodedecyl sulphate polyacrylamide gel elettroforesis (SDS-PAGE) and posses full biological activity as tested by the rescue assay and by MDDCs uptake.
Measurement of IgG anti-Tat is especially valuable in sera and plasma mice immunized with Tat protein.

PRINCIPLE OF THE ASSAY

This assay is based on the microplate enzyme immunoassay technique; Tat protein has been coated onto the wells of the microplate. Revelation step includes a polyclonal anti-IgG HRP-conjugated specific for mouse and ABTS as chromogen.
Sample or standard are pipetted into wells of a microplate pre-coated with Tat protein and pre-blocked; the protein can then capture anti-Tat IgG and, thereby, immobilizing it to the well.

LIMITATIONS OF THE PROCEDURE

FOR RESEARCH USE ONLY

- No drugs have been investigated for assay interference
- Do not use reagents beyond expiration date printed on the kit label
- Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature or kit age can cause variation in binding.

REAGENTS

96 well polystyrene microplate (12 strip of 8 well) coated with Tat protein and blocked with BSA 1%
Plate Cover : 1 adhesive plate sealers
Assay diluent: (Buffer A) 50 mL ready to use, with preservative
Wash buffer concentrate: (Buffer B) 125 mL 10x to dilute to 1250 final volume with distilled water, with preservative.
Conjugated Antibody: Goat Anti rabbit IgG-HRP conjugated 20µl
Color buffer: (Buffer C) 12.5 mL with preservative
1 tablets of ABTS (Buffer C_1) 12.5µl

STORAGE

Maintain the kit at 2-8°C. Do not use kit past its expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm.
- 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 Vials

PRECAUTIONS

The buffer C1 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 antibody used in this assay as no testing can offer complete assurance of freedom from infectious agents.

SAMPLE COLLECTION AND STORAGE

Serum: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. For that reason centrifuge, after clotting, at approximately 1000 x g for 10 min and remove serum.
Plasma samples should be collected in heparin or EDTA and centrifuged at 1000 x g for 10 min; plasma should be removed rapidly and carefully from the red cells after centrifugation.
Storage: If not analyzed shortly after collection, freeze samples in small aliquots (25-50µl) and store them at –80°C. Avoid freeze-thaw cycles.
Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneus before assaying.

REAGENT PREPARATION

Bring all reagents to room temperature prior to use.
96 wells microtiter plates: balance the plate at room temperature for 1 h before use.
Assay diluent: ready to use. Utilize this buffer for sera or plasma dilutions in a clean glass vial.
Wash Buffer concentrate: If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 125 mL of Wash Buffer (Buffer B) concentrate into deionized or distilled water to prepare 1250 mL of Wash Buffer.
Conjugated antibody: Prepare immediately before use. Dilute 1:1000 in buffer A, in a clean glass vial according to the number of wells to be used (100µl / well). Do not keep this dilution for further experiments.
Color buffer: reconstitute immediately before use the ABTS tablet in 12.5 mL of buffer C and mix with 12.5 µl of buffer C_1. The Color buffer is light sensitive. Avoid prolonged exposure to light.

ASSAY METHOD

Bring all reagents and samples to room temperature before use. It's recommended that all sample and standars be assayed in duplicate.

1 Take away the sealing from the plates
2 Dispense the serum at appropriate dilution in buffer A (100 µl / well)
3 Maintain the plates 90 min. at 37°C: cover the plates.
4 Wash the plates five times (in diluted buffer B)
5 Dispense the Goat anti mouse IgG HRP-conjugated diluted 1:1000 in buffer A (100 µl / well)
6 Maintain the plates for 60 min. at 37°C: cover the plates.
7 Wash the plates five times (in diluted buffer B)
8 Dispense the reconstituted color buffer (100 µl/well)
9 Cover the plates. Await the development of the color at room temperature: usually it takes from a minimum of 20 min. to a maximum of 45 min.
10 Read the abs at 405 nm using a microplate reader

QUALITY CONTROL

Each testing laboratory should establish a quality control program to monitor the performance of the Rabbit Anti-TAT IgG Immunoassay.

ASSAY PROCEDURE SUMMARY

– Dispense the serum at appropriate dilutions 100 µl /well
Maintain the plates at 37°C for 90 min.
– Aspirate and wash five times.
– Add 100µl per well of conjugated antibody
Maintain the plates at 37°C for 60 min
– Aspirate and wash five times
– Add 100 µl / well of Color Buffer
Incubate 20-45 min. RT, Read at 405 nm

REFERENCES

– Arya SK, Guo C, Josephs SF, Wong-Staal F. "The trans-activator gene of human T- Lymphotropic virus type III (HTLV-III)" Science. 1985 Jul 5;229(4708):69-73

– Reiss P, Lange JM, de Ronde A, de Wolf F, Dekker J, Debouck C, Goudsmit J: "Speed of progression to AIDS and degree of antibody response to accessory gene products of HIV-1" J Med Virol. 1990 Mar;30(3):163-8

– Ensoli B, Buonaguro L, Barillari G, Fiorelli V, Gendelman R, Morgan RA, Wingfield P, Gallo RC: "Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral *trans* activation" J Virol. 1993 Jan;67(1):277-87

– Chang HK, Gallo RC, Ensoli B "Regulation of cellular gene expression and function by the human immunodeficiency virus type 1 Tat protein" J Biomed Sci. 1995 Aug;2(3):189-202

– Re MC, Furlini G, Vignoli M, Ramazzotti E, Roderigo G, De Rosa V, Zauli G, Lolli S, Capitani S, La Placa M: "Effect of antibody to HIV-1 Tat protein on viral replication in vitro and progression of HIV-1 disease in vivo" J Acquir Immune Defic Syndr Hum Retrovirol. 1995 Dec 1;10(4):408-16

– Fauci AS: "An HIV vaccine: breaking the paradigms" Proc Assoc Am Physicians. 1996 Jan;108(1):6-13. Review.

– Borrow P, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, Nelson JA, Gairin JE, Hahn BH, Oldstone MB, Shaw GM: "Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus" Nat Med. 1997 Feb;3(2):205-11

– Kim DT, Mitchell DJ, Brockstedt DG, Fong L, Nolan GP, Fathman CG, Engleman EG, Rothbard JB: "Introduction of soluble proteins into the MHC class I pathway by conjugation to an HIV tat peptide" J Immunol. 1997 Aug 15;159(4):1666-8

– Chang HC, Samaniego F, Nair BC, Buonaguro L, Ensoli B: "HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region" AIDS. 1997 Oct;11(12):1421-31.

– Zagury JF, Sill A, Blattner W, Lachgar A, Le Buanec H, Richardson M, Rappaport J, Hendel H, Bizzini B, Gringeri A, Carcagno M, Criscuolo M, Burny A, Gallo RC, Zagury D: "Antibodies to the HIV-1 Tat protein correlated with nonprogression to AIDS. A rationale for the use of Tat toxoid as an HIV-1 vaccine" J Hum Virol. 1998 May-Jun;1(4):282-92

– Re MC, Vignoli M, Furlini G, Gibellini D, Colangeli V, Vitone F,La Placa M "Antibodies against full-length Tat protein and some low-molecular- weight Tat-peptides correlate with low or undetectable viral load in HIV-1 seropositive patients" J Clin Virol. 2001 Apr;21(1):81-9

– Wu Y, Marsh JW "Selective transcription and modulation of resting T cell activity by preintegrated HIV DNA" Science 2001 Aug 24;293(5534):1503-6

– Fanales-Belasio E, Moretti S, Nappi F, Barillari G, Micheletti F,Cafaro A, Ensoli B: "Native HIV-1 Tat protein targets monocyte-derived dendritic cells and enhances their maturation, function and antigen-specific T cell responses" J Immunol. 2002 Jan 1;168(1):197-206

– Corinti S, Chiarantini L, Dominici S, Laguardia ME, Magnani M, Girolomoni G: "Erythrocytes deliver Tat to interferon-gamma-treated human dendritic cells for efficient initiation of specific type 1 immune responses in vitro" J Leukoc Biol. 2002 Apr;71(4):652-8

– Dominici S, Laguardia ME, Serafini G, Chiarantini L, Fortini C, Tripiciano A, Brocca-Cofano E, Scoglio A, Caputo A, Fiorelli V, Gavioli R, Cafaro A, Ensoli B, Magnani M: "Red blood cell-mediated delivery of recombinant HIV-1 Tat protein in mice induces anti-Tat neutralizing antibodies and CTL" Vaccine. 2003 May 16;21(17-18):2073-81