HTLV BLOT 2.4

For detection of antibodies to HTLV-I and HTLV-II in serum or plasma samples.

NAME AND INTENDED USE

The GENELABS DIAGNOSTICS (GLD) HTLV BLOT 2.4 is a qualitative enzyme immunoassay for antibodies to HTLV-I and HTLV-II in human serum or plasma samples. This test kit is supplied for research purposes only. It is not intended for use in the diagnosis or prognosis of disease. In particular, this test cannot be used to evaluate blood specimens for the purposes of donor screening or as a confirmatory diagnostic.

INTRODUCTION

The **GLD HTLV Blot 2.4** is an informational research test on serum or plasma samples. The GLD HTLV Blot 2.4 incorporates MTA-1, a unique HTLV-I envelope recombinant protein (rgp46-1), K55, a unique HTLV-II envelope recombinant protein rgp 46-II and GD21, a common yet specific HTLV-I and HTLV-II epitope recombinant envelope protein. Each strip also includes an internal sample addition control to minimize the risk of false negatives due to operational errors.

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips are incorporated with HTLV-I viral proteins derived from native inactivated disrupted viral particles and genetically engineered proteins. Individual nitrocellulose strips are incubated with diluted serum or plasma specimens and controls. Specific antibodies to HTLV-I/II, if present in the specimen will bind to the HTLV-I/II proteins on the strips. The strips are washed to remove unbound materials while antibodies that bind specifically to the HTLV proteins can be visualized using a series of reactions with goat anti-human IgG conjugated with alkaline phosphatase and the substrate, BCIP/NBT.

KIT COMPONENTS

1. **NITROCELLULOSE STRIPS** Available in
Incorporated with HTLV-I viral lysate 18 & 36 strips

and recombinant envelope antigens.
Keep dry and away from light.
1

Trial Version

2. **NON-REACTIVE CONTROL** 1 vial
Inactivated normal human serum (80ul)
non-reactive for anti-HCV, anti-HIV-
1/2, anti-HTLV-I/II and HBsAg.
Contains sodium azide
and thimerosal as preservatives.
3. **STRONG REACTIVE CONTROL I** 1 vial
Inactivated human serum with (80ul)
high titered antibodies to
HTLV-I and non-reactive for anti-HCV,
anti-HIV-1/2 and HBsAg. Contains
sodium azide and thimerosal as
preservatives.
4. **STRONG REACTIVE CONTROL II** 1 vial
Inactivated human serum with (80ul)
high titered antibodies to
HTLV-II and non-reactive
for anti-HCV, anti-HIV-1/2 and HbsAg.
Contains sodium azide and
thimerosal as preservatives.
5. **LYOPHILIZED STOCK BUFFER** 1 or 2 bottles
To be reconstituted in (each to be
reagent grade water. reconstituted
Tris buffer with heat inactivated to 100ml)
animal and non-animal proteins.
Contains thimerosal as preservative.
6. **WASH BUFFER CONCENTRATE (20X)** 1 bottle
Tris with Tween-20 and contains (70ml)
thimerosal as preservative.
7. **CONJUGATE** 1 vial
Goat anti-human IgG conjugated (120ul)
with alkaline phosphatase.
8. **SUBSTRATE** 1 bottle
Solution of 5-bromo-4-chloro (100ml)
-3-indolyl-phosphate (BCIP) and
nitroblue tetrazolium (NBT).
8. **BLOTTING POWDER** 10 packets
Non-fat dry milk (1g each)
9. Incubation trays, 9 wells each. 2 or 4 trays
2

Trial Version

10. Instruction Manual 1 copy
11. Forceps 1 pair
Volume of reagents provided are sufficient for 4 runs.

PRECAUTIONS TO USERS

CAUTION: Handle all assay specimens, positive and negative controls as
potentially infectious agents.
1. Substituting reagents, even between lots, may affect results.
2. FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
3. Do not use kit components beyond the expiry date.
4. Avoid microbial contamination of reagents when opening and removing aliquots
from the original vials or bottles.
5. Gloves and lab coats must be worn.
6. Do not pipette by mouth.
7. Wipe spills quickly and thoroughly with sodium hypochlorite solution.
8. Autoclave all used and contaminated materials at 121°C at 15 p.s.i. for 30 minutes before disposal.
9. It is highly recommended that this assay be performed in a biohazard cabinet.
10. Decontaminate all used chemicals and reagents in sodium hypochlorite solution.
11. We do not recommend re-use of incubation trays.

STORAGE INSTRUCTIONS

A. **Antigen strips**

- Avoid unnecessary exposure of antigen strips to light.

B. **Reagents**

- Store all reagents at 2 - 8 °C.
- Fore best results, dispense reagents while cold and return to 2 - 8 °C storage
as soon as possible.

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CAUTION: Avoid unnecessary exposure of substrate to light.

MATERIALS REQUIRED BUT NOT PROVIDED

Rocking platform *
Pipettor and tips
Aspirator with sodium hypochlorite trap *
56°C water bath [optional]
*** Not required if using Autoblot System 36.**

SPECIMEN HANDLING AND STORAGE (OPTIONAL)

Sera can be inactivated but this is not a requirement for optimal test performance.
Inactivated as follows:
1. Loosen caps of serum containers.
2. Heat serum at 56°C for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis.

We recommend that the sera should not undergo repeated freeze-thaw cycles prior
to testing.

PREPARATION OF REAGENTS

1. **DILUTED WASH BUFFER**
(a) Dilute 1 volume of WASH BUFFER CONCENTRATE (20X) with 19 volumes reagent grade water. Mix well.
2. **BLOTTING BUFFER**
(a) Reconstitute each bottle of LYOPHILIZED STOCK BUFFER with 100ml reagent grade water. Mix well to dissolve. This RECONSTITUTED STOCK BUFFER is stable for 6 weeks if stored at 2-8^{0}C
(b) BLOTTING BUFFER **should be prepared fresh prior to use**.
Add 1 g of BLOTTING POWDER to every 20 ml of the RECONSTITUTED STOCK BUFFER prepared in step 2(a) above. Mix well.
4

Trial Version

3. **WORKING CONJUGATE SOLUTION**
(a) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example 10ul CONJUGATE to 10ml BLOTTING BUFFER.
(b) WORKING CONJUGATE SOLUTION should be **prepared fresh prior to use**.
4. **SUBSTRATE SOLUTION (ready to use)**
(a) Dispense directly the required volume from the bottle. Use a clean pipette. Cap tightly after use.

RECOMMENDED ASSAY PROCEDURE

Note: Aspirate all used chemicals and reagents into trap containing sodium hypochlorite.

1. Using forceps, carefully remove required number of STRIPS from the tube and place numbered side up into each well. Include strips for Strong Reactive and Non-Reactive controls.
2. Add 2ml of DILUTED WASH BUFFER to each well.
3. Incubate the strips for at least 5 minutes at room temperature (25 + 3°C) on a rocking platform. Remove buffer by aspiration.
4. Add 2ml of BLOTTING BUFFER to each well followed by 20ul each of patients' sera or controls to appropriate wells.
5. Cover the tray with the cover provided and incubate for 1 hour at room temperature (25 + 3°C) on the rocking platform.
6. Carefully uncover the tray to avoid splashing or mixing of samples . Aspirate the mixture from the wells. Change aspirator tips between samples to avoid crosscontamination.
7. Wash each strip 3 times with 2ml of DILUTED WASH BUFFER allowing 5 minutes soak on the rocking platform between each wash.
8. Add 2 ml of WORKING CONJUGATE SOLUTION to each well. Cover tray and incubate for 1 hour at room temperature (25 + 3°C) the rocking platform.
9. Aspirate CONJUGATE from the wells. Wash as in step 7.

10. Add 2 ml of SUBSTRATE SOLUTION to each well. Cover tray and incubate for
15 minutes on the rocking platform.

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11. Aspirate the SUBSTRATE and rinse the strips several times with reagent grade
water to stop the reaction.
12. Using forceps, gently remove strips onto paper towels. Cover with paper towels
and dry.
13. Mount strips on worksheet (non-absorbent white paper). Do not apply adhesive
tape over the developed bands. Observe the bands (see interpretation of Results) and grade the results. For storage, keep the strips in the dark.

AMOUNT OF REAGENTS REQUIRED FOR
VARIOUS NUMBER OF STRIPS

Reagents	NUMBER OF STRIPS TO BE USED						
	3	6	9	15	20	27	36
1X Wash Buffer (ml)	60	100	140	240	300	400	520
1X Blotting Buffer (ml)	20	40	60	80	100	120	160
Conjugate (ul)	11	17	23	35	45	59	77
Substrate (ml)	11	17	23	35	45	59	77
Blotting Powder (g)	1	2	3	4	5	6	8

REFERENCE STANDARDS

We recommend that the Non-Reactive Control and both Strong Reactive Controls be
run with assay regardless of the number of samples tested.

1. **NON-REACTIVE CONTROL**

No HTLV-I/II viral specific bands, rpg46-I, rpg 46-II or GD21 should be observed
on the Non-Reactive control strip. The band for the serum control (anti-human IgG) should be visible.

2. **STRONG REACTIVE CONTROL I**

The serum control band and all relevant HTLV-I/II molecular weight bands must
be evident . The relevant HTLV-I bands must be present are p19, p24, gp46, gp46-1 and GD21. Note that the gp46 band is diffused.

3. **STRONG REACTIVE CONTROL II**

The serum control band and all relevant HTLV-I/II molecular weight bands must
be evident . The relevant HTLV bands mist be present are p24, GD21 and rpg46-II.

IDENTIFICATION OF BANDS

The serum control band serves as a check for serum addition in the assay. Absence
of this band indicates that no test serum or conjugate or substrate has been dispensed onto the test strip or other operational errors.

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Locate and identify bands on the strips run with Strong Reactive Controls. These strips are then used to identify bands present on strips used with test specimens.
Serum with antibodies to both viruses although rare, may occur and can also be differentiated based on the above criteria. Banding patterns of such specimens will indicate HTLV-I and HTLV-II positive. Available data demonstrates that the seroreactivity to rgp46-I is specific for HTLV-I and seroreactivity to rgp46-II is specific for HTLV-II.

LIMITATIONS OF THE PROCEDURE

Deviation from the recommended procedure may lead to aberrant results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no express warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied, including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any other purposes. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic loss however caused by the product in the use or in the application thereof.

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Trial Version

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