GENTAUR

GENELABS® DIAGNOSTICS

HIV-1 BLOT 1.3

WESTERN BLOT ASSAY

Instruction Manual

FOR RESEARCH USE ONLY
NOT FOR USE IN
DIAGNOSTIC PROCEDURES

For detection of antibodies to HIV-1 in serum or plasma samples.

NAME

The GENELABS DIAGNOSTICS HIV-1 BLOT 1.3 is a qualitative enzyme immunoassay for antibodies to HIV-1 in human serum or plasma.
This 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 purpose of donor screening, or as a confirmatory diagnostic.

INTRODUCTION

The Genelabs Diagnostics HIV-1 BLOT 1.3 Western Blot is an informational research
test on serum or plasma specimens. The separated specific viral antigens incorporated
onto the strips via electrophoretic and electrotransblot procedures, will also allow for
further delineation of the antibody responses to specific viral proteins. Each strip also
includes an internal sample addition control to minimize the risk of false negatives due
to operational errors and to ensure the addition of samples.

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The nitrocellulose strips are incorporated with separated, bound antigenic proteins from
partially purified inactivated HIV-1 using electrophoretic blotting. Individual nitrocellulose
strips are incubated with diluted serum or plasma and controls. Specific antibodies to

HIV-1 if present in the specimens, will bind to the HIV-1 proteins on the strips. The strips
are washed to remove unbound materials. Antibodies that bind specifically to HIV-1
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 HIV-1 viral lysate. 18 or 36
Keep dry and away from light. strips
2. **NON-REACTIVE CONTROL** 1 vial
Inactivated normal human serum non-reactive (80 ul)
for Hepatitis B surface antigen (HBsAg), antibodies to HIV-1
and HCV. Contains sodium azide and thimerosal as
preservatives.
3. **STRONG REACTIVE CONTROL** 1 vial
Inactivated human serum with high titered antibodies to (80 ul)
HIV-1 and non-reactive for HBsAg and anti-HCV.
Contains sodium azide and thimerosal as preservatives.
4. **WEAK REACTIVE CONTROL** 1 vial
Inactivated human serum with low titered antibodies to (80 ul)
HIV-1 and non-reactive for HBsAg & HCV. Contains
sodium azide and thimerosal as preservatives.
5. **STOCK BUFFER CONCENTRATE (10X)** 1 bottle
Tris buffer with heat inactivated normal goat serum. (20ml)
Contains thimerosal as preservative.
6.. **WASH BUFFER CONCENTRATE (20X)** 1 bottle
Tris with Tween-20. Contains thimerosal as preservative. (70 ml)
7. **CONJUGATE** 1 vial
Goat anti-human IgG conjugated with alkaline phosphatase. (120ul)
8. **SUBSTRATE** 1 bottle
Solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) (100ml)
and nitroblue tetrazolium (NBT).

9. **BLOTTING POWDER** 10 packets
Non-fat dry milk (1g each)
10. Incubation Tray, 9 wells each. 2 or 4 trays
11. Instruction Manual 1 copy
12. 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.

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

CAUTION: Avoid unnecessary exposure of substrate to light.

MATERIALS REQUIRED BUT NOT PROVIDED

Rocker platform *
Pipettors 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.

Inactivate as follows:

1. Loosen caps of serum containers.
2. Heat serum to 56°C for 30 mins 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 of reagent grade water. Mix well.

2. **BLOTTING BUFFER**

(a) BLOTTING BUFFER should be **prepared fresh prior to use**.

(b) Dilute 1 volume of STOCK BUFFER CONCENTRATE (10X) with 9 volumes of reagent grade water. Mix well.

(c) Add 1 g of BLOTTING POWDER to every 20ml of the DILUTED STOCK BUFFER prepared in step 2(b) above. Mix well.

3. **WORKING CONJUGATE SOLUTION**

(a) Prepare WORKING CONJUGATE SOLUTION by diluting CONJUGATE 1:1000 into BLOTTING BUFFER, for example, 5ul CONJUGATE to 5ml 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 - RAPID ASSAY

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, Weak 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 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) on 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.

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 and grade the results. For storage, keep the strips in the dark.

ALTERNATIVE RECOMMENDED PROCEDURE - OVERNIGHT ASSAY

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, Weak 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 of each of sera or controls to appropriate wells.
5. Cover the tray with the cover provided and incubate overnight (16-20 hours) 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 30 minutes at room temperature (25 ± 3°C) on 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.

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 and grade the results. For storage, keep the strips in the dark.

SUMMARY RECOMMENDED ASSAY PROTOCOLS

Reagents Qty Rm Temp
Rapid Assay
Rm Temp

Overnight Assay
Nitrocellulose strip 1 - -
Wash Buffer 2ml 5 mins 5 mins
Blotting Buffer 2ml - -
Specimen 20ul 60 mins Overnight
(16 - 20 hours)
Wash Buffer 3 x 2ml 3 x 5mins 3 x 5mins
Conjugate 2ml 60 mins 30 mins
Wash Buffer 3 x 2ml 3 x 5 mins 3 x 5 mins
Substrate (Ready to use) 2ml 15 mins 15 mins
Distilled Water 2ml - -

Note: All incubations are to be carried out on a rocking platform. Alternatively GENELABS offers an Autoblot System 36 which is designed to perform all Genelabs
Diagnostics Western Blot assays automatically. Please contact your nearest distributor
for more information.

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, Strong Reactive and Weak Reactive controls
be run with every assay regardless of the number of samples tested.

1. **NON-REACTIVE CONTROL**

No HIV-1 specific bands should be observed on the Non-Reactive control strips.
The band for the serum control should be visible (Fig 1c).

2. **STRONG REACTIVE CONTROL**

All relevant molecular weight bands must be evident. Figure 1a provides a guide
to the relative positioning of bands visualised with the Genelabs Diagnostics HIV-
1 Blot 1.3 and permits identification of bands observed for the STRONG REACTIVE CONTROL. The bands are p17, p24, p31, gp41, p51, p55, p66 and
gp120/gp160. Other bands associated with core antigens (ie. p39, p42) may also
be visible. Be careful not to misinterpret these as gp41. The envelope antigens,
gp41, gp120/gp160 appear as diffuse bands as they are typical of glycoproteins.
The serum control band will be visible.

3. **WEAK REACTIVE CONTROL**
Weak bands at p24 and gp120/160 should appear . Some additional weak bands
may or may not be present. The serum control band will be visible (Fig 1b).

INTERPRETATION OF BANDS

The presence or absence of antibodies to HIV-1 in a sample is determined by comparing each nitrocellulose strip to the assay control strips tested with the NONREACTIVE,
STRONG REACTIVE and WEAK REACTIVE controls.
The Figure 1a on Page 11 is suggested as an aid to identify the various bands which
develop on the strip reacted with the STRONG REACTIVE Control.

Molecular Weight Gene	Antigen	Description
gp 160	ENV	Polymeric form of gp41 Broad diffuse glycoprotein
gp 120	ENV	Outermembrane Diffuse glycoprotein
p66	POL	Reverse Transcriptase Discreet band
p55	GAG	Precursor protein Discreet band
p51	POL	Reverse Transcriptase Discreet band just below p55
gp41	ENV	Transmembrane Diffuse glycoprotein
p39	GAG	Fragment of p55 Discreet band
p31	POL	Endonuclease Doublet
p24	GAG	Core protein Broad band
p17	GAG	Core protein Broad band

Some of the different antigens mentioned in the Table above are derived from the same
precursor protein and may have overlapping epitopes. This should be considered when
interpreting the pattern, for example:-

1. It is unlikely to detect gp41 in the absence of gp160 because the gp160 is the
polymeric form of gp41 and the concentration of gp160 is higher than gp41 on the HIV-1 Blot 1.3.
2. The p55 band is generally detected when there is strong reactivity to p24 and/or
p17. The bands seen as p42 and p39 are both GAG fragments and should not be interpreted as gp41 (ENV).
3. The POL bands p66, p51 and p31 are generally detected simultaneously. However the sensitivity of p66 and p31 are greater than p51.
4. HIV-2 cross reactivity is variable but typically shows reactivity with GAG and/or
POL antigens. However, there can be cross reactivity with the gp160 band in some cases, but rarely with gp41.
5. There is also a high molecular weight band around 160KD that is presumed to be
a GAG-POL precursor protein. This is seen with some high titered HIV-2 or

Indeterminate (GAG Reactive Only) sera but the band pattern is a sharp discreet
band which is different from the diffuse band of ENV gp160.

LIMITATIONS OF THE PROCEDURE

Deviation from the recommended procedure may lead to aberrant results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no 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, express or implied, including such
express or implied warranty with respect to merchantability, fitness for use or implied
utility for any purpose. 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 howsoever
caused by the product in the use or in the application thereof.

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Trial Version