

USING ADVICE FOR LDBIO DIAGNOSTICS WESTERN BLOT KITS

This manual aims to guide the LDBIO Diagnostics users in their manipulations, independently of the parameter. It completes the Instructions For Use (IFUs) of each kit and should be followed in parallel. Every step described comes from our own experience in the use of our kits and from the clients' issues we have managed during the last 20 years.

This manual is destined to indicate all the practices that we have validated in order to allow our kits to provide you reproducibility and performance.

The first part is dedicated to users on manual technique, the second is for automate users.

MANUAL MANIPULATION USERS

1. Kit's conditioning

The LDBIO Diagnostics Western Blot (WB) kits are constituted of 3 sub-parts:

- Transfers (12 or 24 strips) kept in a plastic bag
- Liquid reagents
- Positive control

As written in all our IFUs, every liquid reagent with the same batch number can be used together, with all parameters and independently of the parameter. To decrease the risk of bacteriological contamination we also advise you to take the bottles sequentially and **to open only one bottle of each reagent at the same time**, for all parameters.

After you received the kits:

- We advise you to class transfers and reagents by order of arrival, to use the oldest strips and reagents first.
- If the boxes are kept, use them by order of arrival.
- If the boxes are deconditioned, class chronologically transfers and positive controls by parameter, and the reagents by batch number.

2. Equipment use

Pipets must be clean et calibrated, single-use tips changed between each serum. If the pipet touches the sample tube wall, clean it with absorbent paper before the next pipetting.

For the manual incubation wells maintenance (8 wells trays):

- Rinse the manual incubation wells with water. **DO NOT USE BLEACH OR DETERGENTS.**
- Incubation wells, even blackened, can be used a long time before replacement.

Note: Do not use tap water to rinse the incubation wells, as it can contain more or less bleach depending on the season.

Agitation during incubation times is critical and conditions the quality of the results. It is ESSENTIAL that the strips can move freely in their wells: speed and amplitude of rocking must be sufficient. We recommend the use of a rocking platform as below, with a 60 RPM speed:



3. Western Blot test realization

The Western blot technique is based on biological reactions, for which **ambient temperature is critical**. The lower the temperature, the weaker the results. LDBIO Diagnostics tests have been optimized for temperatures comprised **between 21 et 26°C**.

Use of positive controls

The positive control technically validates or invalidates a manipulation. This serum has to show clean bands. From a manipulation to another, the same positive control (same batch number) must be identical (number of bands, intensity).

Note 1: Positive controls with different batch numbers may show different profiles in terms of number of bands, intensity and clearness.

Note 2: From our experience, positive controls are stable. A drop of intensity usually testifies of a manipulation or a contamination (mostly the conjugates) problem.

IT IS ESSENTIAL TO HAVE AT LEAST ONE POSITIVE CONTROL BY MANIPULATION.

Note 3: You can't base your interpretation with a positive control strip which doesn't come from the same transfer: the calibration of the position and the aspect of the bands can slightly vary between two transfers.

Note 4: The use of a positive control strip next to the patient's one (for instance strips #3 et #4) is the best method to determine the position of an unknown band on the patient's strip.

Manipulation plan

Write a manipulation plan with **ALL** the information necessary for the traceability of the test. Each plan must at least indicate the following points:

- Date
- Technician's name
- Manual/automated manipulation
- **Batch number of the transfer**
- **Batch number of the reagents**
- **Batch number of the positive control** (don't forget to add a dedicated strip)
- Incubation times

Note: alone, the batch number of the kit doesn't tell us which reagents have been used for the manipulation. Only the serial number written on the box or the detail of the information listed above can assure the traceability. In the case you need a support, we need to know precisely these elements.

Reagents distribution

- The repeated pipetting of reagents in the original tubes is a critical step to avoid contaminations: it is essential to avoid the formation of bubbles in the pipet tips: the reagents can produce foam, and the bubbles can rinse the body of the pipet leading to a massive bacterial contamination of the reagents.

Note: Pipetting in another tube than the original bottle may be a good idea. In this case, do not refill the reagent in the original bottle after its use.

Washing steps

- Add washing buffer in every well up to half-height. Move the incubation wells by avoiding:
 - Overflows
 - Inter-wells contaminations
 - Flipping of the strips
- Incubate 3 minutes on the agitator.
- Slowly return the incubation box, wells down to eliminate the washing buffer. Strips remain stick to the wells by capillarity. Shake the box to eliminate the last drops.
- Restart this washing twice.

Note: The concentrated washing buffer must be diluted 10 times in DI water. Be careful to **well homogenize the diluted buffer before use.**

Strip distribution

The distribution of the strips and sera must be performed on a cleaned and cleared bench.

- Tip: In the case strips of same number are used in a same manipulation, you can distinguish them by doubling the blue alignment line (cf. picture below). Use a blue BIC pen, and a ruler.



- Detach or cut the strips above the alignment line with a scalpel: do not detach them with your fingers or touch the strip below the alignment line.
- Deliver **1.2mL** of sample diluent in each well.

Note: this 1.2mL volume ensures a sufficient volume for the immersion and incubation of the strip. A lower volume can lead to a bad revelation, a volume too important to a contamination between the wells.

- Distribute the strips according to the manipulation plan established, number of strips towards top. **Strips must be laid down at the surface of the sample diluent: they must float.**
- Let the strips rehydrate for 1-2 minutes then immerse them slowly by moving the incubation wells.

THE COMPLETE AND NATURAL REHYDRATATION OF THE STRIPS IS ESSENTIAL FOR THE GOOD PROGRESS OF THE TEST.

Sera distribution

Sera distribution in the incubation wells must respect the following points:

- Every serum has to be vortexed before distribution, as they might have been frozen.
- Check the volume set on the pipet (10 or 25µL, cf. IFUs of each kit).
- Wet the tip by a first pipetting of the serum, release, then second pipetting.
- Distribute the serum in the sample diluent on the side of the strip. Release all the serum in the well, release the piston only after the tip is out of the well (the presence of some air bubbles after the distribution validates that all the serum has been released).
- Homogenize by slowly shaking the incubation tray 3-4 times **after each distribution.**

- Incubate the samples **90 min** on a rocking plate.
- Wash 3 times 3 min with the diluted washing buffer.
- Deliver **1.2mL** of conjugate and incubate **60 min** with agitation.
- Wash 3 times 3 min with the diluted washing buffer.
- Deliver **1.2mL** of substrate and incubate **60 min** with agitation and protected from direct sun light.
- Stop the reaction by washing 2 times with DI water. Strips can remain in water for several hours but have to be taken out and dried (on absorbent paper for instance) before their interpretation.

Interpretation

- Wait for the strips to be completely dry: they become clearer after drying.
- Once dried, organize the strips by parameter, transfer and number order, spaced by 1-2mm maximum, and aligned between them thanks to the alignment line of each strip.

Note: This “reconstitution” of the transfers after revelation (the transfer is the original unit of production of the WB) greatly refines the quality of the interpretation, allowing the specific bands to be followed throughout the transfer.

- Use the positive control strip to determine the presence and position of the specific bands potentially present on every patient’s strip.
- If the strips are associated to the patient’s file, copy the references of the manipulation plan with the strips. Keep the manipulation plan to ensure manipulation traceability.

Note: You can find an example of the profiles obtained with our positive controls for every parameter in the “MYLDBIO” space on our website www.ldbiodiagnostics.com.

AUTOMATE USERS

1. Kit's conditioning

The LDBIO Diagnostics Western Blot (WB) kits are constituted of 3 sub-parts:

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- Liquid reagents
- Positive control

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After you received the kits:

- We advise you to class transfers and reagents by order of arrival, to use the oldest strips and reagents first.
- If the boxes are kept, use them by order of arrival.
- If the boxes are deconditioned, class chronologically transfers and positive controls by parameter, and the reagents by batch number.

2. Equipment use

Pipets must be clean et calibrated, single-use tips changed between each serum. If the pipet touches the sample tube wall, clean it with absorbent paper before the next pipetting.

It is better if you can reserve your automate to the sole and only use of LDBIO Diagnostics kits. The use of reagents from another manufacturer may interact and alter the results. Such a mixed utilization, if necessary, will have to be validated, under full lab responsibility, and the automate profusely rinsed before using LDBIO kits.

Maintenances have to be done, they are described in their respective IFUs. In the case of use of an Autoblot or an Euroblot/Dynablot automate, maintenances consist in:

- Daily rinsing first with the diluted washing buffer, then with DI water
- Monthly decontamination with soda and calibration of the pumps
- Annually, tubes and pumps have to be changed. This maintenance is usually done by the supplier.

THESE MAINTENANCES ARE ESSENTIAL TO THE GOOD PERFORMANCE OF THE AUTOMATE AND TO GUARANTEE THE RESULTS.

For the manual incubation trays maintenance (20, 30, 44 or 48 wells trays depending on the automate):

- Rinse the incubation trays with DI water. **DO NOT USE BLEACH OR DETERGENTS.**
- Incubation trays, even blackened, can be used a long time before replacement.

Note: Do not use tap water to rinse the incubation trays, as it can contain more or less bleach depending on the season.

3. Western Blot test realization

The Western blot technique is based on biological reactions, for which **ambient temperature is critical**. The lower the temperature, the weaker the results. LDBIO Diagnostics tests have been optimized for temperatures comprised **between 21 et 26°C**.

Use of positive controls

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Note 3: You can't base your interpretation with a positive control strip which doesn't come from the same transfer. Indeed, the calibration of the position and the aspect of the bands can slightly vary between two transfers.

Note 4: The use of a positive control strip next to the patient's one (for instance strips #3 et #4) is the best method to determine the position of an unknown band on the patient's strip.

Manipulation plan

Write a manipulation plan with **ALL** the information necessary for the traceability of the test. Each plan must at least indicate the following points:

- Date
- Technician's name
- Manual/automated manipulation
- **Batch number of the transfer**

- **Batch number of the reagents**
- **Batch number of the positive control** (don't forget to add a dedicated strip)

Note: alone, the batch number of the kit doesn't tell us which reagents have been used for the manipulation. Only the serial number written on the box or the detail of the information listed above can assure the traceability. In the case you need a support, we need to precisely know these elements.

Reagents distribution

- For a better reagent management, you must only use the necessary volume for the tests with a small safety extra, by using the bottles provided, or 50mL Falcon/Corning tubes. As they are conical, you can save more reagents.
- If the volumes are not sufficient, you can complete with a new reagent bottle **with the same batch number**.
- **DO NOT REFILL A REAGENT IN THE ORIGINAL BOTTLE AFTER USE:** from our experience, you may keep the remaining volumes in the tubes at +4°C for 2 weeks. Discard them, clean the tubes (no bleach nor detergent) and use new reagents every 2 weeks.

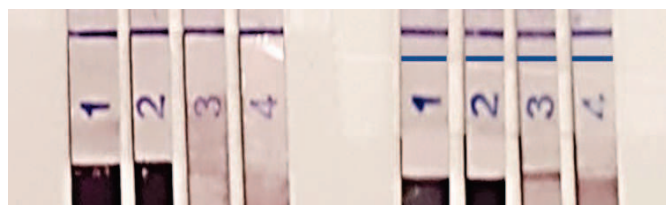
Note: LDBIO will provide additional reagents at your request to compensate this loss.

Note: The concentrated washing buffer must be diluted 10 times in DI water. Be careful to **well homogenize the diluted buffer before use**.

Strip distribution

The distribution of the strips and sera must be performed on a cleaned and cleared bench.

Tip: In the case strips of same number are used in a same manipulation, you can distinguish them by doubling the blue alignment line (cf. picture below). Use a blue BIC pen and a ruler.



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- Distribute the strips according to the manipulation plan established, number of strips towards top. **Strips must be laid down at the surface of the sample diluent: they must float.**
- Let the strips rehydrate for 1-2 minutes then immerse them slowly by moving the incubation tray/wells.

THE COMPLETE AND NATURAL REHYDRATION OF THE STRIPS IS ESSENTIAL FOR THE GOOD PROGRESS OF THE TEST.

Sera distribution

Sera distribution in the incubation wells must respect the following points:

- Every serum must be vortexed before distribution, as they might have been frozen.
- Check the volume set on the pipet (10 or 25µL, cf. IFU of each kit).
- Wet the tip by a first pipetting of the serum, release, then second pipetting.
- Distribute the serum in the sample diluent on the side of the strip. Release all the serum in the well, release the piston only after the tip is out of the well (the presence of some air bubbles after the distribution validates that all the serum has been released).
- Homogenize by slowly shaking the incubation tray 3-4 times **after each distribution.**
- Start the automate:
 - o Sample incubation **90 min** with agitation.
 - o 3 washing steps with diluted washing buffer.
 - o Distribution of **1,2mL** of conjugate, and **60 min** incubation with agitation.
 - o 3 washing steps with diluted washing buffer.
 - o Distribution of **1,2mL** of substrate, and **60 min** incubation with agitation, protected from light.
- Reaction is stopped by washing 2 times with DI water. Strips can remain in water for several hours but must be taken out and dried (on absorbent paper for instance) before their interpretation.

Interpretation

- Wait for the strips to be completely dry: they become clearer after drying.
- Once dried, organize the strips by parameter, transfer and number order, spaced by 1-2mm maximum, and aligned between them thanks to the alignment line of each strip.

Note: This “reconstitution” of the transfers after revelation (the transfer is the original unit of production of the WB) greatly refines the quality of the interpretation, allowing the specific bands to be followed throughout the transfer. Use the positive control strip to determine the presence and position of the specific bands potentially present on every patient’s strip.

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