Instruction for Use

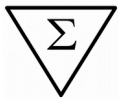
AMODIA DetectLine

Basic *plus*

Molecular Test System
for the detection of

Amplification Products

based on the AMODIA®-LFD *plus*
(LFD: Lateral-Flow Dipstick)



100

REF:
ADL-B02

AMODIA Bioservice GmbH

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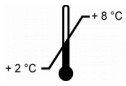
Explanation of Symbols

Symbol	Explanation
	Expiry date
IVD	In Vitro Diagnostic Medical Device
LOT	Batch code
REF:	Catalog number
	Storage conditions

Symbol	Explanation
	Consult Instruction for Use
	Consult attended documents
	Package size
	Manufacturer
	Only for evaluation purposes

Description of the Test

The AMODIA DetectLine Basic *plus* is able to detect two amplification products at the same time, each labeled with two labels. The product detected on the bottom test line has to be labeled with biotin and FITC/FAM. The product detected on the middle test line has to be labeled with digoxigenin and FITC/FAM. The top line is an assay control (v. fig 2).
False-positive results may be avoided if the amplification reaction has only one labeled primer per target followed by a subsequent hybridization with a labeled probe.

This test-kit does not contain materials to generate those detectable amplification products.

Material Supplied, Storage and Stability

Components	Cat-No.	Content (100 tests)	Preparation	Storage	Shelf Life / Stability
Detection					
Lateral Flow Dipsticks	LFD04	4 vials a 25 pcs.	ready to use	2 - 8°C*	Until expiration date; 60 days after opening
Chromatographic Buffer	ChB02	4 vials a 10 ml	ready to use	2 - 8°C	Until expiration date; 60 days after opening

* : LFD vial must be locked tightly ! Storage with opened LFD vial reduces the stability of the LFDs.

Important note:
Expiration dates should not be exceeded.

Reagents Required

None

Laboratory Instruments and Materials Required

- Adjustable pipettes for 10 µl and 1.000 µl (three different sets for extraction, amplification and detection)
- Sterile pipette tips with contamination protection (filter tips)
- Microwell plate

Warnings and Precautions

All reagents of this test kit are strictly intended for the specified diagnostic use only. Use by staff, who is especially trained in those methods.

Please adhere strictly to the sequence of processing steps provided by this protocol.

Store all reagents in the originial vials at the temperatures indicated on the respective labels. Do not interchange kit components of different lots and assays. Do not use kit components beyond their expiration dates.

Stick to the safety rules for handling kit reagents and sample materials. Especially be aware of the following precautions:

- do not eat, drink or smoke
- wear safety clothes and gloves
- avoid contact with reagents and sample material

Some reagents contain preservation substances against microbial growth, so avoid contact with skin and/or mucous membranes.

Empty vials could be discarded with the normal laboratory waste.

Test Characteristics

Distributor: AMODIA Bioservice GmbH
Order-No.: ADL-B02
Package size: 100 reactions
Delivery: from stock in Braunschweig, Germany
Storage: view "Material Supplied, Storage and Stability"

Test time and procedure:

Detection and read-out	approx. 15 minutes

Product Detection

Attention:
- Do not interchange components of different lots.
- Perform the product detection by all means at an area different from the amplification area. (**post-amplification area**).

Lateral-Flow Dipsticks (v. fig. 1) are used for the detection of amplifaction products. They consist (from the bottom) of an area for the chomatographic buffer (white), a spot of sample application (purple), a membrane and an absorption area (white). With the exception of the application area the whole strip is covered by a foil. The strip can be touched at the covered areas. Remarks should be made only on the foil above the absorption area.

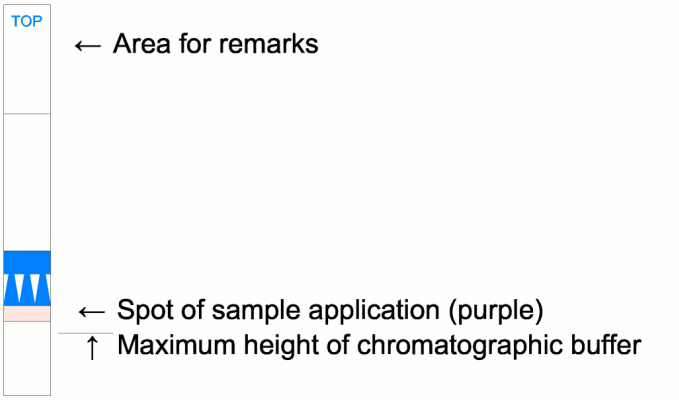


Fig. 1: Set-up of the Lateral-Flow Dipstick

1.1 Detection

	Steps for detection
1.	Take the required number of LFDs from the vial and label them. Only touch the areas covered with foil and use the white area at the end of the LFD for labeling. Close the LFD vial tightly.
2.	For each sample dispense **150 µl chromatographic buffer ChB02** in single reaction vials or in wells of a (clean) microwell plate.
3.	Pipet **5 - 10 µl** of the solution containing amplification products onto the application area (purple) at the edge of the foil. A bleeding of the liquid is normal. Incubate for **1 min**.
4.	Dip the LFDs with the membrane into the chromatographic buffers prepared in step 2 until the application area and the membrane besides the lines are fully discolored (at least 15 minutes). The control line must be visible. Do not read the result before the end of the incubation time. The lines are stable and can be read later.

1.2 Interpretation of Results

Please note that it is NOT recommended to use both test lines for target. It is recommended to use the upper test line (middle line) as an inhibition control. This allows to distinguish a negative test result by no target ("true negative") from an inhibited amplification ("false negative").

1.	**Three** lines are visible: the test lines and the assay control line **Note**: Even a faint test line has to be interpreted as positive. Consider the PCR negative control for a comparison. If needed the test must be repeated for confirmation. Depending on the concentration positive results may be visible even before the entire incubation time is over.	The detection of two amplification products is **positive**.
2.	**Two** lines are visible: one test line and the assay control line **Note**: In case the positive test line is an inhibition control, then this result is true negative for the other test line (target).	The detection of the respective visible amplification product is **positive**.
3.	**Only one** line gets visible at the position of the assay control line **Note**: The application area should have no violet staining anymore before reading-out the result.	The amplification of both amplification products is **either negative**, **or** the amplification has been **inhibited**.

*: This interpretation is only correct if the middle line is used as an inhibition or internal control !

The result of a test is only valid if the control line of every sample is stained.
The **positive and negative controls** for the complete test run have to be correct in order to validate the results.
The **positive control** must show a test line **clearly visible**.
The PCR **negative control** must show **no visible test line**. If a stained line occurs, the analysis of **all** the samples tested in parallel must be repeated. If only the signal of the extraction control is positive, a different procedure has to be applied (v. Trouble-Shooting).

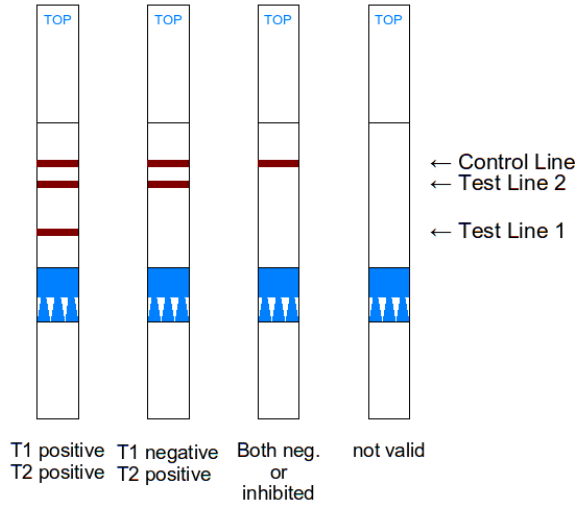


Fig. 2: Interpretation* of the Lateral-Flow Dipstick

The AMODIA DetectLine Basic *plus* only gives a qualitative result. The intensity of the stained test lines has no direct relation to the number of double-labeled amplification products present in a positive sample.

Trouble-Shooting

Problem	Possible cause	Recommendation
No assay control line visible	a) Wrong or disfunctional chromatographic buffer ChB02	Use new chromatographic buffer.
	b) Test strips expired	Use new test strips.
	c) Wrong storage of the test strips (moist strips)	**Store at 2 - 8°C. Close the vials tightly !**
All samples and the positive control show a negative signal	a) Amplification not successful	Check the amplification products on a agarose gel.
	b) Labeling of the amplification products not successful	Check the reagents of the labeling.
All samples and controls show a positive signal	a) Contamination of the PCR reaction	a) Decontaminate the PCR work place and all pipettes. Use new gloves and new pipet-tips from a new box.
	b) Chromatographic buffer contaminated with target organism and/or amplification product	b) Use fresh chromatographic buffer
	b) Other reagents contaminated with target organism and/or amplification product	c) Use fresh reagents

Method / Test principle

The detection of the two double-labeled amplification products is performed using an immuno-chromatographic assay on a Lateral-Flow Dipstick. Both types of amplification products bind with one label to an antibody which is immobilised on gold particles (gold conjugate). With the diffusion of the chromatographic buffer all gold particles diffuse through the membrane. This membrane is prepared with three lines of different capture molecules. At the first line (bottom) the capture molecules bind to the biotin label of the first amplification product. So gold particles bound to these amplification products are accumulated at this site, thus forming a visible line. At the second line (middle) the capture molecules bind to the digoxigenin label of the second amplification product. As at the first line gold particles bound to these amplification products are accumulated here forming a visible line. Gold particles without amplification product move on. The third line (top) gets visible if the gold conjugate is still intact. This serves as an assay control for the correct performance of the Lateral-Flow Dipstick.
The sensitivity of the AMODIA Lateral-Flow Dipstick is slightly better than a DNA detection with an agarose gel stained with Ethidiumbromide, which is known to detect approx. 10 ng DNA.

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