

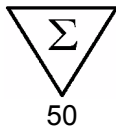
Instructions for Use

AMODIA

**easyFlow®
Yeast & Mould**

Molecular test system
for the detection of

RNA of Yeasts and Moulds



REF:
AEF-YM1



Distributed by:

AMODIA Bioservice GmbH

Rebenring 31
D-38106 Braunschweig
Germany




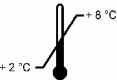
Tel.: +49 (0) 531-260 17 64
Fax: +49 (0) 531-260 17 66






E-mail: info@amodia.de
Internet: <http://www.amodia.com>

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Explanation of Symbols

Symbol	Explanation
	Expiry date
	<i>In Vitro</i> Diagnostic Medical device
	Batch code
REF:	Catalog number
	Storage conditions

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

Introduction

The easyFlow® detection method is based on the hybridization of ribosomal RNA with labeled probes. Several regions of the ribosomal RNA are characteristic for a species like a human fingerprint. In dead or not replicating cells the RNA is degraded quickly, thus only viable replicating cells are detected. For usage of the easyFlow® assay no amplification techniques (e.g. PCR) are necessary.

Description of the Test

The test-kit AMODIA easyFlow® Yeast & Mould is intended for microbial quality control. It detects the RNA of viable and replicating yeasts and moulds directly from an enrichment culture.

The easyFlow® Yeast & Mould test-kit does not use amplification to detect yeasts and moulds. It consists of the moduls: a) hybridization of probes to 18S rRNA and b) detection of the formed complex on Lateral-Flow Dipsticks (LFDs).

Materials Supplied, Storage and Stability

Components	Cat.- No.	Content (50 tests)	Preparations	Storage	Stability / Shelf life
Pre-treatment					
Suspension Buffer (white cap)	SB02	1 vial a 5 ml	ready to use	room temperature	Until expiration date; 60 days after opening
Hybridization					
Hybridization Buffer (white cap)	HBP02	1 vial a 1,8 ml	ready to use	room temperature	Until expiration date; 60 days after opening
Probe Mix (purple cap)	PME02	1 vial a 300 µl	ready to use	4 - 8°C	Until expiration date; 60 days after opening
Detection					
Lateral Flow Dipsticks	LFD01	2 vials a 25 pcs.	ready to use	4 - 8°C	Until expiration date; 60 days after opening*
Chromatographic Buffer (blue cap)	ChB01	1 vial a 10 ml	ready to use	4 - 8°C	Until expiration date; 60 days after opening

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

ⓘ Important note:

Expiration dates should not be exceeded.

Laboratory Instruments and Materials Required

- Thermal cycler with led heater
- Adjustable pipettes for 10 µl, 100 µl and 1.000 µl
- Rack made of plastic or aluminium, suitable for the PCR tubes
- Microwell plate
- Centrifuge for 1,5 ml reaction tubes
- PCR tubes compatible with the thermal cycler
- Sterile pipette tips with contamination protection (filter tips)

Warnings and Precautions

All reagents of this test-kit are strictly intended for the specified *in vitro* diagnostic use only.

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

Store all reagents in the original 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 materials

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

Empy vials could be discarded with the normal laboratory waste.

Test Characteristics

Distributor: AMODIA Bioservice GmbH
 Order-No.: AEF-YM1
 Package size: 50 reactions
 Delivery: from stock in Braunschweig, Germany
 Storage: see „materials supplied, storage and stability“
 Sample material: no manufacturer`s warranty!

Every sample material has to be evaluated by the operator!

	sample material
Required sample material:	1 ml liquid enrichment culture
Analytic specificity:	<u>highly specific for:</u> Eucaryota coverage of 88 % in the domain Eucaryota

Test time and procedure:

Hybridization set-up	approx. 20 minutes
Hybridization reaction	approx. 35 minutes
Detection and read-out	approx. 15 minutes
Total test time ⁺	approx. 70 minutes

+ : with heating- and cooling rates of 1,5°C/s

Examples for Liquid Enrichment Cultures

It is necessary to perform an enrichment culture prior to the detection reaction. Procedures already validated should be used unchanged for the preparation of these enrichment cultures. This is why there are no reagents for this included in the kit.

The volume of the enrichment culture can be adapted. The following example demonstrates the required adjustments of the volumina.

Steps to prepare the Enrichment Culture	
1.	Pipet 1 g product (~ 1 ml) into a sterile reaction tube. Use a fresh pipette tip or transfer pipette for each sample.
2.	Q.S. to 10 ml with an appropriate inactivation solution* .
3.	Mix samples for at least 60 s until a homogeneous solution has formed. Incubate the solution for 10 to 30 min at room temperature.
Alternative A: 100 ml enrichment culture	
4.a	Pour 90 ml nutrient solution** into a suitable flask.
5.a	Add 10 ml of the sample solution and incubate for 14 – 24 hours at 30°C .
Alternative B: 10 ml enrichment culture (lower sensitivity possible!)	
4.b	Pour 9 ml nutrient solution** into a suitable flask (e.g. 50 ml Falcon tube).
5.b	Add 1 ml of the sample solution and incubate for 14 – 24 hours at 30°C .

*: solution that inactivates preservatives in the tested sample

** : see note

i Note:

Nutrient solutions with ingredients for inactivation of preservatives (e.g. Eugon LT broth) should be used according to manufacturer`s specifications.

Assay Conditions

⚠ Important notes:

- Open and close the reaction vials individually.
- Change gloves immediately, if contaminated.
- Open the reaction tubes carefully in order to avoid aerosols. If possible centrifuge for a few seconds.
- Hybridized products (RNA-probe conjugate) should be treated as an important source of potential contamination.
- Do not interchange components of different lots.

1. Modul: Hybridization

1.1 Pre-treatment

For RNA extraction centrifuge 1 ml enrichment culture for 10 min at 13'000g. The supernatant is discarded completely. The sediment is mixed with 100 µl suspension buffer. Now the samples are ready for the hybridization reaction.

⚠ Warning:

It is necessary to remove as much culture medium as possible after centrifugation. Otherwise false-positive results may occur.

Steps for RNA Extraction	
1.	Prepare and label the required number of sterile 1,5 ml reaction tubes.
2.	For every sample transfer 1 ml enrichment culture into single reaction tubes. For each tube use a new pipette tip. Close the tubes individually.
3.	Centrifuge the samples at 13'000g for 10 min.
4.	Remove the supernatant completely from the sediment. For each tube use a new pipette tip. Close the tubes individually.
5.	With a new filter tip for every sample pipet 100 µl suspension buffer into the reaction tubes. Open tubes individually and close them immediately after pipetting.
6.	Suspend the sediment in the buffer by pipetting several times up and down or vortex for 10 s.

1.2 Preparation of Reaction Mixture

Into each sterile reaction tube (PCR-tube) pipet **30 µl hybridization buffer** and **5 µl probe mix**. Add **10 µl lysed sample** from 1.1.

For more than one test reaction the preparation of a mastermix is recommended. Therefore the volumes for a single reaction have to be multiplied with the factor of reactions (samples + controls + 1).

Hybridization Mixture	Volume per reaction	Example: mastermix for 10 reactions
Reagent	Volume per reaction	Factor = 11
Hybridization Buffer	30,0 µl	330,0 µl
Probe Mix	5,0 µl	55,0 µl
		pipet 35 µl into each of the 10 reaction tubes, discard the remaining mixture
Enrichment culture or colony in buffer	10,0 µl	add 10,0 µl individually

Steps for Hybridization	
1.	Prepare and label the required number of sterile PCR tubes. Process all pipetting steps without interruption. It is recommended to process a negative control in every test run.
2.	Prepare mastermix (see above).
3.	Pipet 35 µl mastermix into the reaction tubes. Close the reaction tubes.
4.	Add 10 µl sample solution from 1.1 to the reaction mixture. For each tube use a new pipette tip. Open tubes individually and close them immediately.
5.	Negative controls contain no further material.

1.3 Hybridization Reaction

Place the samples in the thermal cycler and start the hybridization program.

i The assay was developed using a Primus thermal cycler from PEQLAB. If a different thermal cycler is used an adaption of the program may be necessary. If you have any questions, please contact the manufacturer of the test-kit.

Programming of the thermal cycler for the hybridization reaction

Set lid heating to 110°C.

Denaturation	temperature* 15 min* at 95°C
Annealing	ramp** 0,1°C/s to 50°C
	temperature* 15 min* at 50°C
Cooling	temperature* 3 min* at 20°C

*: The specified times given are the reaction times **without** the time required for cooling and heating!

: means: slow cooling from **95°C to 50°C, following a step at 50°C for 15 min.

The total duration time of the hybridization program is about 45 min.

2. Modul: Product Detection

⚠ Attention:

- Do not interchange components of different lots.
- Hybridized RNA-probe conjugates** are an important **source of contamination** and should be pipetted and disposed with care.

Overview

Lateral-Flow Dipsticks (v. Fig. 1) are used for the detection. They consist of an area of sample application (purple), a membrane and an absorption area (white) With exception of the application area the whole strip is covered by a tape. The strip can be touched at the covered areas. Remarks should be made only on the tape above the absorption area. Hybridization products are applied directly onto the Lateral-Flow Dipstick.

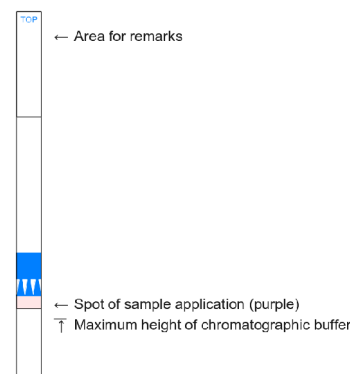


Fig. 1: Layout of the Lateral-Flow Dipsticks

2.1 Detection

Steps for Detection	
1.	Prepare the required number of LFDs and label them. Only touch the areas covered with tape and use the white area at the end of the LFD for labeling. Close the LFD vial tightly.
2.	Pipet 150 µl chromatographic buffer into single vials or wells of a microplate for each reaction.
3.	Pipet 10 µl of the hybridization reaction close to the edge of the tape marked with triangles onto the free membrane part of the LFD (v. Fig. 1). A bleeding of the liquid is normal.
4.	Dip the LFDs with the area without tape into the chromatographic buffer prepared in step 2. Allow an incubation of 20 min . Make sure that the application area is discolored. The control line must be visible for interpretation of results. The lines are stable and can be read later.

2.2 Interpretation of Results

1.	<p>Two lines are visible: test line and control line (v. Fig. 2)</p> <p>⚠ Notes: Even a faint test line has to be interpreted as positive. For a comparison the negative control should be considered. If necessary the test must be repeated for confirmation. Positive results may be visible even before the entire incubation time is over.</p>	The detection of RNA of yeasts or moulds is positive .
2.	<p>Only one test line is visible at the location of the control line.</p> <p>📌 Note Do not interpret the results until the incubation time is over.</p>	The detection of RNA of yeasts or moulds is negative .

The result of the test is only valid if the control line of every sample is visible.

The **negative controls** for the complete test run have to be correct in order to validate the results. If a negative control shows a visible test line, the analysis of **all** samples tested in parallel must be repeated.

The dried test strips could be adhered to an evaluation sheet and stored.

The test-kit gives only a qualitative result. The intensity of the stained test line has no direct relation to the number of cells present in a positive sample.

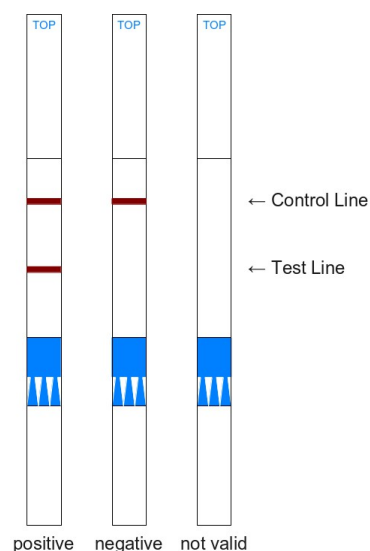


Fig. 2.: Interpretation of results

Method / Test Principle

1.) Sample Preparation

The preparation of an enrichment culture is necessary to ensure that enough RNA molecules for detection are available. Only replicating microorganisms produce RNA, so that only viable microorganisms are detected. Nucleic acids (e.g. DNA) of non-replicating or dead cells are not detected. A centrifugation step concentrates the cells of microorganisms eventually present in the samples and removes residue of the enrichment culture. The sediment is suspended.

2.) Hybridization

A part of the suspended sample solution is mixed with the probe mix. During the following temperature controlled hybridization reaction the cells are lysed. Labelled probes hybridize to the released ribosomal RNA. This RNA-probe complex is detected directly on the LFD.

3.) Detection

The detection is performed using an immuno-chromatographic assay on a Lateral-Flow Dipstick. With this device the hybridization product is detected.

The hybridized complex of RNA and probe binds to an antibody, which is immobilised on gold particles. With the diffusion of the chromatographic buffer the complex also diffuses through the membrane. This membrane is prepared with two lines of different capture molecules. At the first line only gold particles are bound which are also linked to the hybridization product. This binding increases the local concentration of the gold, thus forming a visible line. Gold conjugates without hybridization products diffuse further and bind at the second line. Here the line gets visible if the gold conjugate is still intact. This serves as a control for the correct performance of the Lateral-Flow Dipstick.

Trouble-Shooting

Problems	Possible cause	Recommendation
No growth in enrichment culture	a) Inappropriate growth temperature b) Inappropriate nutrient solution c) Inactivation solution does not work	a) Check optimal growth temperature b) Use nutrient media (e.g. Sabouraud) instead of selective media c) Use freshly prepared inactivation solution or try different solution
No control line visible (upper line)	a) Chromatographic buffer wrong or dysfunctional b) Test strips expired c) Wrong storage of test strips	a) Use new chromatographic buffer b) Use new test strips c) Store at 2 - 8°C
All samples and controls show a positive result.	a) Contamination of hybridization reaction b) Chromatographic buffer is contaminated.	a) Clean bench and pipettes, check reagents. b) Use new chromatographic buffer
Positive control shows a negative signal	a) No or wrong hybridization buffer or probe mix added b) Hybridization failed c) Inhibition by tested product	a) Repeat hybridization, check reagents b) Check hybridization program c) Repeat test, use appropriate inactivation solution or dilution

Attachment

- Template LFD documentation
- Protocol at a glance