milenia biotec

HybriDetect
RPA basic Kit

Recombinase Polymerase Amplification (RPA)
for fast nucleic acid amplification

HybriDetect **RPA basic Kit** | REF **MGHRB 1** | Σ **96 reactions**

IFU / REF MGHRB 1 / REV A / 2026-08-11

Note: Significant changes are indicated by dotted lines in the margin.
A change history can be found at the end of the manual.

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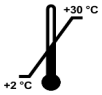
Explanation of Symbols

Symbol	Explanation	Symbol	Explanation
	*Manufacturer*		Temperature limit 2 - 30 °C
	Use-by date		Consult *instructions for use* or consult electronic *instructions for use*
	*Batch Code*		Contains sufficient for <n> reactions/ tests
	*Catalogue number*		Unique Device Identification

Warnings and Precautions

- Store all reagents at 2-30 °C in their original containers, unless otherwise indicated on the label.
- The expiration date of all components must be observed. Do not use components past the expiration date.
- Keep LyoBead tubes strictly sealed until usage. Do not use damaged tubes.
- The disposal of waste materials must be carried out according to current local regulations.
- For professional users.

Safety Data Sheets (SDS)

SDS are available on request or alternatively on the relevant product page on our website www.milenia-biotec.com.

Materials Supplied, Storage and Stability

REF MGHRB 1

Materials Supplied

REF	Amount	Content	Reactions à 50 µl
MGHRB 1	96 x cake 3 x 1,0 ml 3 x 0,1 ml 96 x caps	LyoBead RPA basic 2X RPA Reconstitution Buffer X 20X RPA Reaction Initiator Caps for LyoBead	96

Storage and Stability

Components	Description	Preparation	Storage	Shelf Life
LyoBead RPA basic (REF MGLHRB)	Freeze-dried RPA-Mix for 50µl **Note:** Protect from moisture. Open strips only just before use.	Ready-to-use	2-30 °C	Until EXP
2X RPA Reconstitution Buffer X (REF MGBHRB)	Reconstitution and Reaction Buffer for RPA Master Mix	Ready-to-use	2-30 °C	Until EXP
20X RPA Reaction Initiator (REF MGIHRB)	Initiator for starting RPA amplification process	Ready-to-use	2-30 °C	Until EXP
Caps for LyoBead (REF MGCHRB)	Strip of caps for reaction tubes (PP, highly transparent, flat caps, suitable for qPCR)	Ready-to-use	2-30 °C	Until EXP

Materials Required but not Supplied

- Pipettes
- Nuclease-free water
- *Recommended*: Heating block
- *Recommended*: Tabletop centrifuge

Intended Use

This Milenia GenLine HybriDetect RPA basic Kit (REF MGHRB 1) contains components for a recombinase polymerase amplification reaction with the purpose of amplifying DNA.

The kit is intended for use as a development platform. It is for research use only (RUO) and is not intended for diagnostic procedures and/or commercial applications.

Description

The **Milenia GenLine HybriDetect RPA basic Kit** is a high-performance, ready-to-use solution for rapid isothermal DNA amplification. Operating at a constant temperature between **37 °C and 42 °C**, it eliminates the need for thermal cycling while enabling sensitive DNA amplification and detection within **10–30 minutes**. This fast turnaround time makes it an ideal choice for time-sensitive molecular testing workflows. Supplied in a **lyophilized, pre-formulated format**, the master mix is stable during ambient shipping and storage, eliminating the need for cold chain logistics. Its robust formulation facilitates reliable use in mobile, and field-based environments where refrigerated storage may not be available. The master mix performs consistently across a broad range of sample types, including crude or minimally processed samples, ensuring reliable amplification even under challenging conditions. Thanks to its exceptional robustness and flexibility, the Milenia GenLine HybriDetect RPA basic kit is well suited for diverse molecular biology applications, including life-science research, veterinary and agricultural testing, food and environmental monitoring, and other workflows requiring rapid, reliable isothermal DNA amplification.

Basic Protocol

1. Set up your RPA-reaction mix as shown below:

Component	Volume	Final Concentration
LyoBead RPA basic	1 cake	1X
2X RPA Reconstitution Buffer X	25 µl	1X
Primers and Probes	Variable	Variable
Template	Variable	Variable
Nuclease free water	add up to 47.5 µl	
Transfer the complete volume of the mix to a reaction tube of your choice, then add (into the tube lid): *Alternatively, keep the mix in the original tube and use the provided lid.*		
20X RPA Reaction Initiator	2.5 µl	1X
Total volume	**50 µl**	

2. Close the tube and briefly spin down the Reaction Initiator[1].

3. Incubate your reaction. Recommended reaction conditions: 37–42 °C for 10–30 minutes[2]

4. Evaluation: Evaluate your assay via agarose gel electrophoresis or lateral flow strip.

Note [1]: The reaction starts immediately after the 20X Reaction Initiator enters the mix. To prevent premature initiation of the amplification reaction, keep the RPA Reaction Initiator separate from the RPA Master Mix.

Prepare the complete reaction mixture in the reaction tube without adding the Initiator. Pipette 2.5 µl of the 20X RPA Reaction Initiator onto the inside of the tube lid, taking care not to mix it with the reaction mixture at this stage.

Close the tube securely and briefly centrifuge it in a mini centrifuge to combine the Initiator with the reaction mixture and initiate the reaction.

Note [2]: Optimization of the amplification conditions may be required depending on the target.

Assay Development Guide - Primer Design

Although PCR primers can work as well, the design of specific RPA primers will lead to higher sensitivity and shorter reaction times. The following list is a short guideline on how to design your RPA primers:

- **Length**: RPA primers are longer than PCR primers. The recommended primer length is 30-35 bp but primers up to 45 bp can work too.
- **Length of amplicon**: For efficient amplification, design amplicons between 80 and 200 bp. The amplicon length should not exceed 400 bp.
- **Melting temperature**: For RPA the melting temperature is irrelevant. Primer annealing is driven by the recombinase-primer-complex which is independent of the melting temperature of the primer.
- **GC-content**: The GC content should be between 30-70 %.
- **Sequence**: Avoid repeating sequences as well as the repeat of mononucleotides like "AAAAA".
- **Label incorporation for lateral flow evaluation**: Labels (such as FITC/FAM, biotin, etc.) must be incorporated at the 5´end of the primer to allow elongation by the polymerase.
- **Primer dimers**: Check for primer dimers in-silico and in-vitro

Note: With increasing primer length, the risk of secondary structures will rise which decreases the sensitivity of the assay. Too short primers will reduce the reaction speed and will lower the sensitivity as well.

Assay Development Guide – Enhanced Specificity

If the selected primer pair does not provide sufficient target specificity, an additional target-specific probe compatible with endonuclease IV (Nfo) recognition should be designed. This may be necessary, especially when used in combination with lateral flow test strips.

For the use of Endonuclease IV, a special Nfo probe is needed (see Figure below). This probe contains tetrahydrofuran (THF), an internal abasic nucleotide which replaces a nucleotide. Additionally, it consists of a C3-spacer at the 3´end and an additional label (FITC/FAM or biotin) at the 5´end when combined with lateral flow evaluation. The THF is the binding site for Endonuclease IV which will cleave the Nfo probe if the probe is fully hybridized to its complementary target sequence.

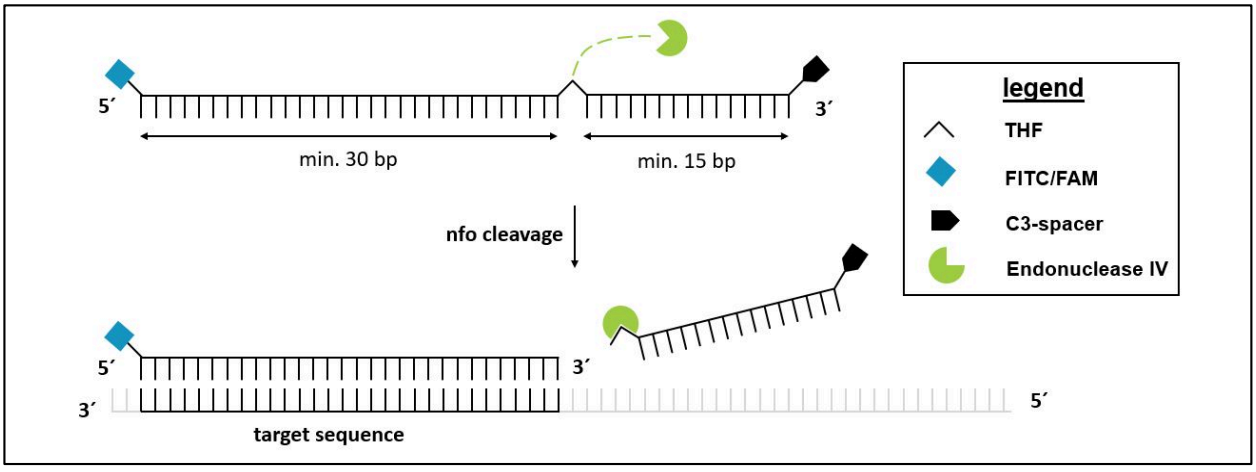


Figure 1: Structure of a Nfo probe for the combination of RPA and lateral flow. The probe can also be labelled with biotin instead of FITC/FAM. Endonuclease IV (Nfo) cleaves nfo probe after completely binding to the target sequence.

Note: Please make sure to avoid dimer formation of the labelled primer and probe, especially if you plan lateral flow evaluation. If the sequences of the primers match partially, they will hybridize and form dimers. Those dimers would carry both a FITC/FAM and biotin label which would be detected as positive by the HybriDetect lateral flow strips. See chapter Troubleshooting for more information on how to prevent dimerization and how to test your designed primers in lab.

Assay Development Guide – Lateral Flow Evaluation

Combine the speed and sensitivity of RPA with the simplicity of lateral flow detection for rapid, reliable, and easy-to-interpret results without the need for complex laboratory equipment. The RPA Kit is directly compatible with our HybriDetect rapid lateral-flow tests, so you have all the components you need in one place.

Preliminary work:
The only thing you need to do to interpret the RPA results using our test strips is to label your primers. Therefore, see the Primer Design chapter above. RPA is a highly processive amplification method. In order to avoid the detection of non-specific RPA products via lateral flow, it is highly recommended to add an additional specificity generating step to the RPA. See therefore chapter Enhanced Specificity above.

Sample Application:
The sample application depends on what product of HybriDetect strip you choose. RPA reactions in general contain components that can harm the antibodies of lateral flow strips, therefore please follow the instructions below. You can either use method a or b.

Lateral Flow Kit	REF	Sample Application
HybriDetect	MGHD 1	a. Add 1 µl of your RPA mix directly to the sample application pad (SAP) of the test strip. Then add or deposit the strip in 50-100 µl of the kit specific running buffer. b. Dilute your reaction mix 1:10 with the kit specific running buffer. Deposit the strip in 50-100 µl of your diluted RPA solution.
HybriDetect Cassette	MGHC 1	a. Add 1 µl of your RPA mix directly to the sample application window of the test cassette. Then add 50 µl of the kit specific running buffer. b. Dilute your reaction mix 1:10 with the kit specific running buffer. Add 50 µl of your diluted RPA solution to the sample application window.
HybriDetect 2T	MGHD2 1	a. Dilute your RPA reaction mix 1:5 with the kit specific running buffer. Add 1 µl of your dilution directly to the sample application pad (SAP) of the test strip. Then add or deposit the strip in 50-100 µl of the kit specific running buffer. b. Dilute your reaction mix 1:20 with the kit specific running buffer. Deposit the strip in 50-100 µl of your diluted RPA solution.

Evaluation:

The minimum incubation time of our test strips is 2 minutes. We recommend an incubation time of 5 minutes for good results. The incubation time should not exceed 15 minutes.

The evaluation (by eye, picture, etc.) should be done immediately after the run is finished. During the drying process of the membrane (after the run has finished) some gold particles can agglomerate at the test lines and form a slight false-positive band after several minutes. After drying the result is invalid.

Note: For further background information see the IFU of the corresponding lateral flow kit.

Troubleshooting

- **Contamination**

  To ensure reliable results, take appropriate precautions during reaction setup to prevent contamination with unwanted RNA, DNA, or nucleases.

  - Use separate, clean work areas for sample preparation and reaction setup.
  - Always wear fresh gloves and use sterile reaction tubes and aerosol-resistant filter tips.
  - Use only nuclease-free water and reagents free from contaminating RNA and DNA.
  - Include a no-template control (NTC) in every assay to monitor for potential contamination.

- **Primer Dimers (LFA)**

  The formation of dimers is a major issue for all amplification related assays (PCR, RPA, LAMP). If the sequences of the primers match (partly), they will hybridize and form dimers. Those dimers would have both, a FITC/FAM and a biotin label which would be shown as positive on the test line by the HybriDetect strip. To avoid dimerization there are several programs to check sequences in silico. We recommend testing the primers for dimerization experimentally before your first experiments to exclude false positive signals through primer dimerization. To check for primer dimers, follow this protocol:

  1. Add an equal amount of your primers to a reaction tube. We recommend a primer concentration of 1 pmol/µl each.
  2. Mix the solution.
  3. Incubate for 5 min at room temperature.
  4. Add 2 µl of the primer mixture to the Milenia HybriDetect strip and deposit the strip in 80 µl chase buffer (MGCB) in an upright position for 5-15 min.

  The formation of a test line will show you the presence of primer dimers. Sometimes dimers will not show a straight visible line. Instead, they will form dots on the left and right side of the test strip (Fig. 2A). This also indicates the presence of primer dimers. If your primers form dimers, you must redesign them. You can rule out dimer formation if no signal is generated on the test line (Fig. 2B).

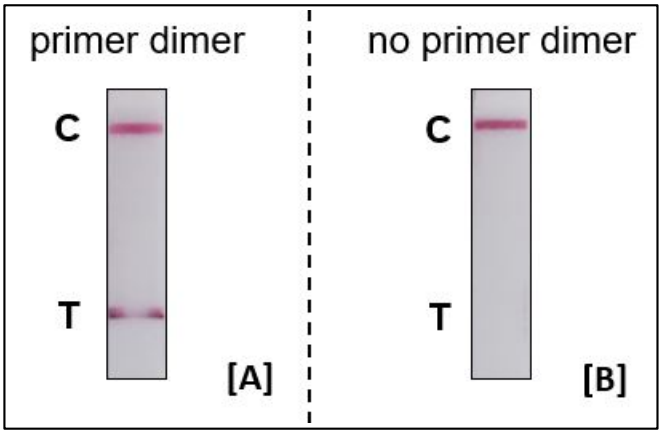


Figure 2: Evaluation of a dual labelled primer pair forming dimers (A) and a dual labelled primer pair without forming dimers (B) with the Milenia GenLine HybriDetect test strip (MGDS).

Limitation of Use

For Research Use Only. The product supplied by Milenia Biotec GmbH is intended exclusively for research purposes. Milenia Biotec GmbH makes no representations or warranties, express or implied, including but not limited to warranties of merchantability, fitness for a particular purpose, or suitability for in vitro diagnostic (IVD) applications or any other specific use.

It is the sole responsibility of the customer to ensure compliance with all applicable laws and regulations and to obtain any necessary approvals from the relevant regulatory authorities before using the product.

This product is not intended for administration to humans or animals and must not be administered to either.

Legal Disclaimer

The purchase of this product does not grant any license to perform patented or otherwise protected methods or applications. It is the sole responsibility of the customer to determine whether the intended use of the product requires any third-party intellectual property licenses and, if necessary, to obtain the appropriate license(s) from the respective rights holder(s).

Additional Products Available

REF	Product Name	Content		Tests	Description
MGHD 1	Milenia GenLine HybriDetect	2 x 50 2 x 10 ml	HybriDetect Dipsticks (MGDS) HybriDetect Assay Buffer (MGCB)	100	Universal lateral flow **dipsticks** with **one** test band (biotin) and one control line.
MGHD2 1	Milenia GenLine HybriDetect 2T	2 x 50 2 x 10 ml	HybriDetect 2T Dipsticks (MGDS2A) HybriDetect Assay Buffer (MGCBB)	100	Universal lateral flow **dipsticks** with **two** different test bands (biotin and digoxigenin) and one control line.
MGHC 1	Milenia GenLine HybriDetect Cassette	4 x 5 1 x 10 ml	HybriDetect Test Units (MGSHC) HybriDetect Assay Buffer (MGCB)	20	Universal lateral flow **cassette** with **one** test line (biotin) and one control line.
HLHSK 1	HybriDetect LAMP Hot Start Kit	1.25 ml 0.125 ml 1 ml	HybriDetect LAMP Hot Start Mix (2x) HybriDetect LAMP Fluorescence Dye (20x) HybriDetect PCR grade water	100	HybriDetect LAMP Kit enables detection of as little as 3 target DNA molecules in a short time.
HLHSP 1	HybriDetect LAMP Hot Start Polymerase	8000 U 2 x 1.25 ml 3 x 1.7 ml	HybriDetect LAMP Hot Start Polymerase (8 U/µl) HybriDetect LAMP Buffer (10x) HybriDetect LAMP Enhancer (5x)	8000 U	The HybriDetect LAMP Hot Start Polymerase is a recombinant protein, representing a large fragment of the B. stearothermophilus DNA Polymerase expressed in E. coli cells.
HLK 1	HybriDetect LAMP Kit	1.25 ml 0.125 ml 1 ml	HybriDetect LAMP Mix (2x) HybriDetect LAMP Fluorescence Dye (20x) HybriDetect PCR grade water	100	HybriDetect LAMP Kit enables detection of as little as 5 target DNA molecules in a short time
HLP 1	HybriDetect LAMP Polymerase	8000 U 2 x 1.25 ml 3 x 1.7 ml	HybriDetect LAMP Polymerase (8 U/µl) HybriDetect LAMP Buffer (10x) HybriDetect LAMP Enhancer (5x)	8000 U	The HybriDetect LAMP Polymerase is a recombinant protein, representing a large fragment of the B. stearothermophilus DNA Polymerase expressed in E. coli cells.
MYILABR 1	myilab research - Lateral Flow Reader	1 x	myilab research device	-	Lateral flow reader for the evaluation of Milenia GenLine HybriDetect test strips and cassettes.

Contact

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Change History

Date	Revision	Cause of Revision
2026-08-11	A	Creation of the instructions for use (IFU)

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