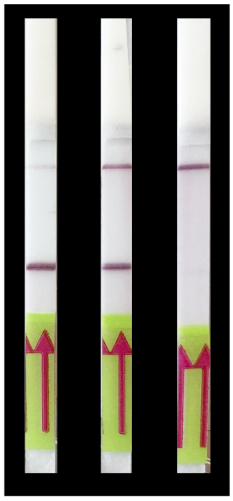
CRISPR/Cas-based Detection Methods and HybriDetect: Universal Test Strips - Individual Readout

Milenia HybriDetect

CRISPR/Cas-based detection methods like SHERLOCK or DETECTR are generally compatible with a rapid, easy-to-handle and -interprete Lateral Flow Readout. The universal dipstick HybriDetect is the most cited Lateral Flow Device for CRISPR/Cas dependent nuclease assays. more information

Lateral Flow - Detection Mechanism

The HybriDetect test strip is able to give information about the status of a so called reporter. A reporter is a short single stranded DNA- or RNA-molecule which carries terminal labels: Biotin and FAM or FITC. Successful target recognition of the CRISPR/Cas-complex triggers collateral nuclease activity of the Cas-Protein. Cas-dependent reporter cleavage leads to the seperation of the terminal labels. The HybriDetect platform is able to differentiate between intact and cleaved reporter.

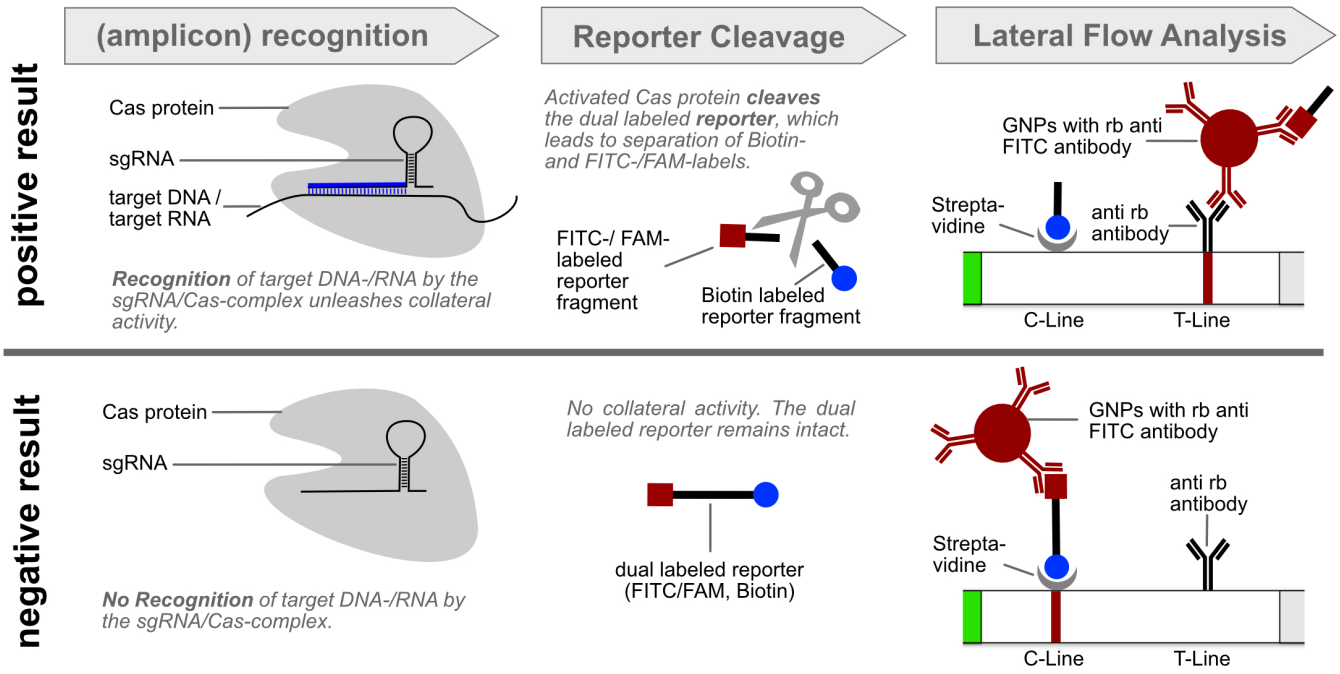


Features of the Test

- *universal Lateral Flow Device*
- *2 to 10 min to your individual Lateral Flow-based result*
- *easy to handle*
- *intuitive readout*
- *compatible with multiple amplification techniques*
- *no further equipment necessary*

Lateral Flow - Interpretation

During LFA, intact reporter is retained at the control-Line (C). The upper test-Line (T) is not visible (negative test result). Cleaved reporter leads to an increasing amount of C-Line-overflowing goldconjugate, which is retained at T. Therefore positive results are characterized by an increasing intensity of T and decreasing intensity of C. more information

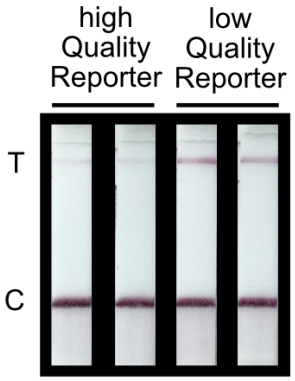


Reporter-Type and -Quality

Diagnostically relevant Cas-proteins show characteristic cleavage preferences. Therefore the reporter-design strongly depends on the choosen nuclease assay type. Nevertheless reporter quality is crucial for succesful CRIPSR/Cas-based-LFA.

Poor quality will result in incomplete T-Line elimination for negative controls, which makes intuitive interpretation difficult.

- use HPLC purified reporters
- protect the reporter for assay-independent degradation
- ssRNA-reporters are sensitive to RNases
- long-term storage at higher concentrations
- avoid repetitive freezing and thawing
- avoid secondary structures or self-dimerization of reporter



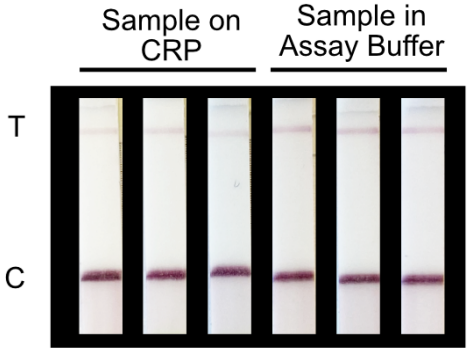
The following table gives a brief overview of frequently used reporters (link for references)

Cas-protein	Reporter-Type	Reporter-Sequence	Ref.
LwaCas13a	ssRNA	5' / 6-FAM / poly-U (≥10) / Biotin / 3'	1,2
LwaCas13a	ssRNA	5' / 6-FAM / RURURURURURURURURURURURURURU / Biotin / 3'	3
LwaCas13a	ssRNA	5' / 6-FAM / MARARURGRGRCMAMARARURGRGRCMA/ Biotin / 3'	4,5,6
LbCas12a	ssDNA	5' / 6-FAM / TTATT / Biotin / 3'	7
LbCas12a	ssDNA	5' / 6-FAM / TTATTATT / Biotin / 3'	8
LbCas12a	ssDNA	5' / 6-FAM / TTATTTTATTTATT / Biotin/ 3'	9

Sample Application

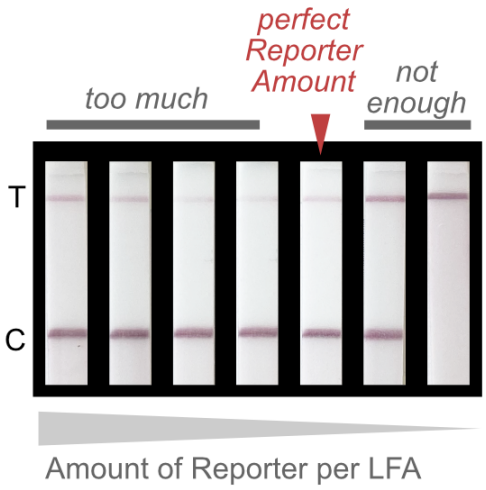
The way the sample is applied to the HybriDetect can influence the readout quality. Mostly frequently, the HybriDetect is used as a dipstick which is directly dipped into a mixture of sample and assay buffer.

But Lateral Flow Assays can benefit from the direct application of the sample to the conjugate release pad (CRP). A volume of 10 µL is optimal to prewet the CRP and allow a concentrated interaction of the goldconjugate with the sample, before lateral flow starts. As a result the flow behavior of multiple strips will be more even and comparable.



Reporter Concentration

Find the correct reporter amount for sufficient T-Line elimination. Defining a certain reporter concentration is important for an easy-to-interprete readout and sensitive LFA-performance.



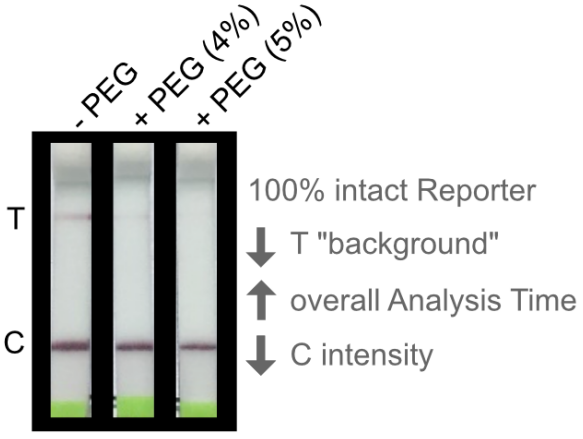
- Pretesting of your specific reporter is inevitable
- Pretest different batches / lots of reporter or HybriDetect
- Approx. 0.2 – 5.0 pmol per LFA should be practicable, according to our experience
- Avoid too much reporter: background on T in negative samples
- Avoid too much reporter: insensitive LFA performance
- Avoid too little reporter: background on T in negative samples
- Use lowest possible amount of reporter with sufficient T-Line elimination

Assay Buffer Modification

The HybriDetect-Kit contains a universal assay buffer which allows a satisfying performance of all relevant LFD-components. Nevertheless, the assay buffer is to be understood as a recommendation. Due to the universal character of the HybriDetect-Kit, the assay buffer is not optimized for a specific application, including reporter-based nuclease assays. Therefore, modification of the universal assay buffer is one of the most underrated tools in HybriDetect-associated analysis.

EXAMPLE: Polyethylenglycol (PEG)

Increased viscosity of the buffer slows down lateral flow speed. Moreover interaction time of mobile and stationary phase of LFD is increased. This can be benificial for better accumulation of reporter at C and thus result in improved T-Line elimination in negative controls.



The Addition of PEG 6000 should give an idea what impact the assay buffer has on the overall detection system. *Be creative and modify the assay buffer for your individual purpose.*

Lateral Flow Analysis-time:

The LFA-time is important for result interpretation. Increased incubation time leads to increased T-Line intensity. Determine the assay-specific optimal Lateral Flow Analysis time to achieve the optimal discrimination between positive and negative results.

- C- and T-Line intensity changes significantly in the first 5 minutes of Lateral Flow
- LFA-time should be clearly defined
- 2 to 5 minutes are commonly used
- Recommendation: documentation after 3 minutes (with standard assay buffer)
- Avoid analysis time deviations especially when handling multiple strips at a time

Handling of Multiple Samples

Positive results are mostly determined by comparison to a negative control. Therefore, it is crucial to create comparable results between different samples. Try to establish a strategy for an accurate and comparable Lateral Flow Assay.

- Every sample (strip) should be treated identically
- Simultanious documentation of multiple strips is recommended
- Try to use the 12-strip-dip-template for handling multiple strips at the same time
- Adding the sample to the CRP improves flow behavoir – more consistent LFA

To minimize handling related artefacts, use 12-strip-dip-template

Digital References

Check out following links if you are interested in more background information.

1. Diagnostic CRISPR tools and techniques (Article)
2. Lateral Flow Readout for CRISPR/Cas-based Detection Strategies (Article)
3. Product: HybriDetect-Universal Lateral Flow Assay Kit
4. Instruction for use: HybriDetect
5. Citation-List 2020: HybriDetect
6. How to perform a HybriDetect-Lateral Flow Assay (Youtube Video)

Please contact Milenia Biotec GmbH for further questions or suggestions.

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Good luck for your Lateral Flow Assay