Application Bulletin 001

Introducing PCRD Nucleic Acid Lateral Flow Immunoassay with Recombinase Polymerase Amplification Compatibility

Part number: PCRD3Kit / Revision A V1.7

PCRD is a nucleic acid lateral flow immunoassay incorporating antibodies which capture and allow visualisation of double-stranded amplification products containing selected binding partners, FITC (or FAM) and biotin, as well as DIG and biotin. This simple method of amplicon detection takes only 10 minutes, and the result is detectable by eye due to an aggregation of carbon particles at the capture lines.
PCRD is a rapid, safe and sensitive alternative to ethidium bromide staining of agarose gels.

Benefits of PCRD

- Compatible with Recombinase Polymerase Amplification (RPA)
- Two test lines enable detection of multiple analytes
- Fully compatible with TwistAmp® nfo kits
- Excellent signal-to-noise ratio
- Improved sensitivity and specificity over Milenia Hybridtech lateral flow strips
- Easy to use; fewer steps than Milenia Hybridtech lateral flow strips
- Results provided in ≤10 minutes

Applications

Due to their versatility, nucleic acid detection assays can be used in a number of applications. Examples include:

- Meat speciation testing
- Food and drink authenticity
- Rapid disease detection – veterinary, human and plant.

PCRD kit contents

50 x PCRD nucleic acid detection lateral flow assays.
5 x extraction buffer (10 mL)
1 x instruction for use (PCRD nucleic acid detector assay IFU)

How to order

PCRD kits can be ordered from https://www.abingdonhealth.com/other-products/nucleic-acid-detection-pcrd/
For more information: orders@twistdx.co.uk, techsupport@twistdx.co.uk

Using PCRD with TwistAmp® nfo

The PCRD has two test lines (anti-FAM and anti-DIG) and a control line, allowing detection of two independent nucleic acid targets. The control line shows that the PCRD is working as expected (see figure 1)

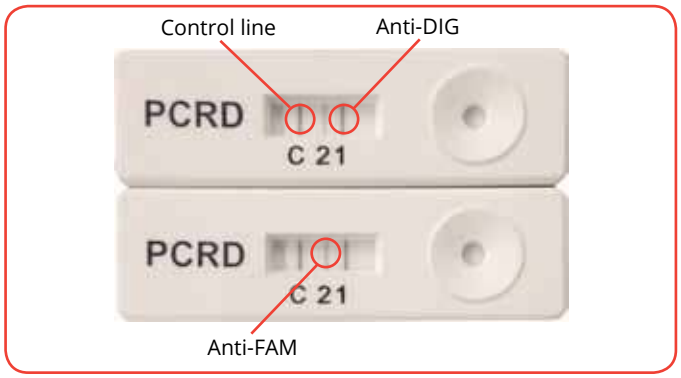

Figure 1

Primer and probe design

Primers and probes for lateral flow detection of TwistAmp® nfo reactions should be designed similarly to that described in detail in the TwistAmp® combined instruction manual and the TwistAmp® assay design manual with the exception that for some targets different labels may be required on the primers and probes. The visible signal is generated using colloidal carbon conjugated to anti-biotin antibody. Therefore, amplicons should be labelled using DIG and biotin (for detection at test line 1) or FAM and biotin (for detection at test line 2).

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We recommend labelling your nfo probe with biotin and your reverse primers with either DIG or FAM, depending on which line you wish to detect your target (see fig. 1).

Note for existing Milenia Hybridtech users

For existing customers using Milenia Hybridtech (one line strips), there is no need to change your existing primers and probes, they are compatible with PCRD. However, for customers using the Milenia Hybridtech 2T two line test strips, the FAM labelled oligonucleotide in your DIG-FAM primer/probe pairs will need to be replaced with a biotin labelled oligonucleotide (no other changes to the sequence should be required).

Detection of RPA products on PCRD

A generic protocol for detection of RPA products on the PCRD is presented; better sensitivity and specificity for particular targets may be obtained by altering the dilution of amplicon in step 2. If optimisation of the dilution conditions is required, it is suggested that the total volume of amplicon/buffer applied to the sample port is in the range of 75 - 100µl. Volumes lower than this are not sufficient to run the test, whilst volumes >100µl can flood the strip membrane and limit test sensitivity significantly.

1. Perform TwistAmp® nfo reactions as described in the TwistAmp® nfo quick guide.
2. Dilute 5ul of amplicon into 70ul of PCRD extraction buffer supplied with the kit (1/15 dilution).
3. Lay the PCRD on a level surface and apply the whole 75ul of buffer/amplicon to the sample port of the PCRD.
4. Leave for up to 10 minutes for the signal to develop.

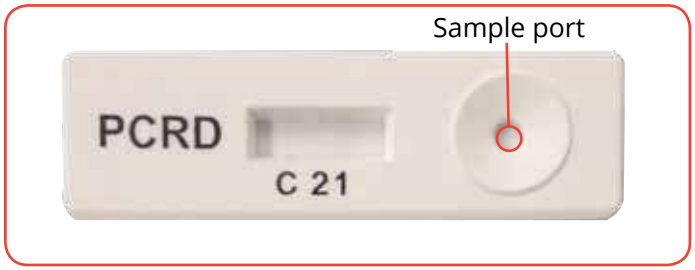

Figure 2

NB: Signals should not be read after 10 minutes as faint false positive test lines can appear after prolonged incubation.
Tests in which the control line does not appear should be deemed invalid, regardless of any signal that appears at either of the test lines. If invalid results are observed repeat the test.

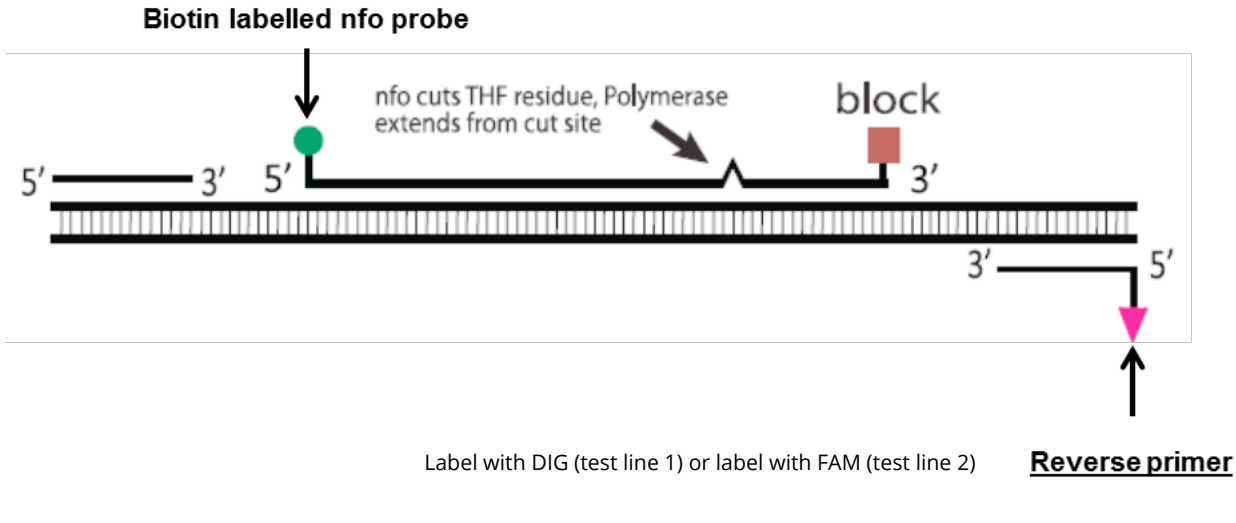

Figure 3. Detect your target

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