# Pro-Detect™ Rapid HA Competitive Assay Kit

**Catalog Numbers** A38509

**Pub. No.** MAN0017617 **Rev.** A.0

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Contents and storage

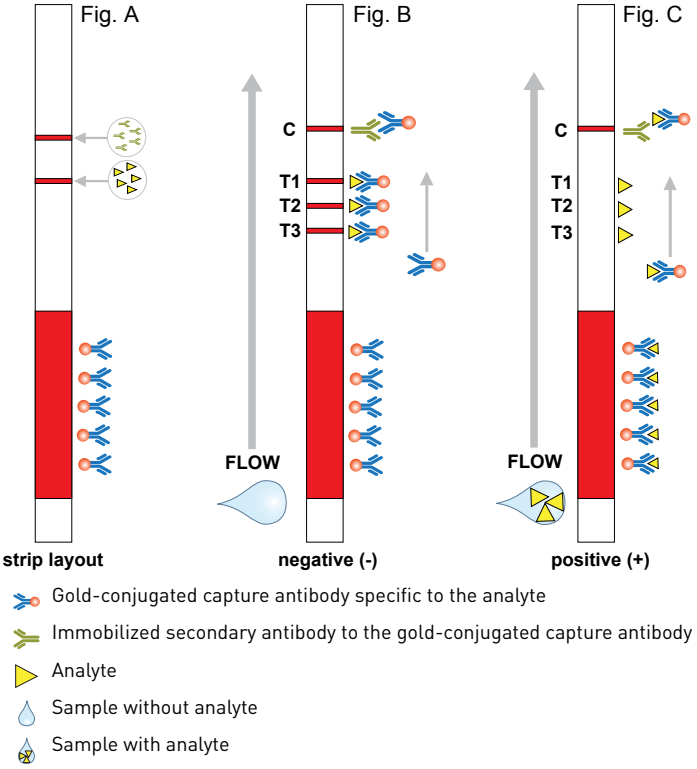
| Product | Cat. No. | Contents | Storage |
|---|---|---|---|
| Pro-Detect™ Rapid HA Competitive Assay Kit | A38509 | Pro-Detect™ Rapid HA Competitive Assay Strips, 10 strips<br>Pro-Detect™ Rapid Assay Dilution Buffer, 15 mL | Store at 4°C. Do not freeze.<br>After opening, store unused strips in the enclosed container containing desiccant. |

## Product description

The Pro-Detect™ Rapid HA Competitive Assay Kit is a 10-minute dipstick lateral-flow assay to detect HA-tagged proteins in cell culture and lysates during protein expression or in purified protein preparations. The assay is performed by simply applying the lateral flow strip into the sample of properly diluted tissue culture supernatant or lysate and visualizing via a *loss* of red bands in the test line section. A red control line will appear in both positive and negative results. In the competitive lateral flow assay, HA-tagged protein antigens immobilized on the membrane as 3 distinct test lines. A control antibody is immobilized as the control line. The gold-conjugated capture antibodies, specific to HA, are embedded in the sample pad (Fig. A).

In a negative result where no HA-tagged protein is present or concentration of HA-tagged protein is below detectable levels, the gold-conjugated capture antibodies will bind to the HA-tagged protein antigen embedded on the test lines and form 3 visible red lines. In both positive and negative tests, the gold-conjugated anti-HA capture antibodies will bind to the control antibodies at the control line (Fig. B).

In a positive test where the sample contains HA-tagged proteins, the gold-conjugated antibodies embedded in the sample pad will bind to the available HA-tagged proteins in the sample and therefore not bind to the HA-tagged protein antigens immobilized on the test lines. As the concentration increases, the number of test lines will decrease until all test lines disappear. The concentration of the HA-tagged proteins is inversely related to the number of test lines appearing on the strip (Fig. C).




## Material preparation

Because the competitive lateral flow assays structure has an inverse relationship with signal loss, there is no true upper limit for the assay; however, dilution is recommended in order to preserve samples. The recommended working range of the HA 4-Line competitive lateral flow assay is 4 µg/mL to 20 µg/mL of a purified HA control protein. The competitive lateral flow assays can detect down to 1 µg/mL, but loss of band intensity may be less distinguished. As there is no upper limit, the lateral flow strip will continue to show positive results at concentrations greater than 20 µg/mL, visualized by complete loss of the test lines (test lines closest to the sample will disappear first). It is not recommended to use at concentrations less than 1 µg/mL.

**Note:** Proper sample dilution is essential for optimal results. Concentration ranges are based upon the concentration of the tagged protein of interest in the sample.

## Sample dilution

If sample concentration is known, dilute the sample with Pro-Detect™ Rapid Assay Dilution Buffer to a concentration of 10 µg/mL. For unknown starting sample concentrations, dilute mammalian cell lysates at 1:4 dilution and bacterial lysates at 1:20.

For example, to make a 1:20 dilution, add 7.5 µL of sample (cell supernatant or lysate) to 142.5 µL of Pro-Detect™ Rapid Assay Dilution Buffer and vortex or pipette up and down to mix.

## Perform test

**Note:** Perform all test at room temperature. To avoid condensation on the strips, allow the package to warm to room temperature for 15 minutes prior to removing the strips from the bag.

1. For each strip, add 150 µL of diluted sample to a microtiter plate or test tube.
2. Insert the lateral flow strip with arrow facing downward into the sample and wait 10-15 minutes for the color bands to appear.
3. Remove the strip(s) from the sample after 10-15 minutes (20 mins max.) of processing time.

   A positive test will result in a red band at the control location and at the test location. Images may be acquired by photograph (camera, cell phone) or imaging equipment. Lateral flow assays may be further saved by placing in notebooks.

After results are obtained, if signal appears weak, perform a lower dilution. A weak signal can result from a high concentration of tagged protein in the sample. The additional dilution will bring the concentration into the acceptable working range. If upon detection with the second dilution no test line is observed, the original test performed was at a concentration below the recommended working range. When the concentration of actual tagged protein is not known in mammalian cell lysate, dilute 1:2; for a bacterial lysate, dilute 1:5.

## Troubleshooting

| Observation | Possible cause | Recommended action |
|---|---|---|
| All 3 test lines detected after applying sample. | Sample did not contain protein tag. | Verify correct assay strip is used. |
| | | Verify presence of protein tag via alternative method (e.g., ELISA or Western blot). |
| Low intensity test lines. | Sample was below recommended concentration range. | Dilute samples as indicated in the Perform test section and re-test using a new lateral flow strip. |
| No control line detected. | Lateral flow strip was not sufficiently submerged in the sample. | Insert test strip fully into sample well and ensure enough volume is present in the sample well to fully cover the white application tip of the lateral flow strip. |
| | Sample contained an interfering substance. | Confirm additional lysis and extraction reagents are within the recommended ranges (see the Additional Information section). |

## Additional information

Lateral flow assays are highly robust assays that can withstand many commonly used detergents, buffers, salts, and other lysis reagents. A list of commonly used reagents and the effective compatible concentrations are provided in the table below.

**Table 1** Assay reagent compatibility.

| Salts/Buffers | | Detergents | |
|---|---|---|---|
| **Substance** | **Compatible Concentration** | **Substance** | **Compatible Concentration** |
| NaCl | 0.25 M | SDS | 0.2% |
| Urea | 0.4 M | Triton™ X-100 Detergent | 1% |
| RIPA Buffer | Undiluted | CHAPS Detergent | 1% |
| B-PER™ Bacterial Protein Extraction Reagent | Undiluted | **Misc. Reagents and Solvents** | |
| M-PER™ Mammalian Protein Extraction Reagent | Undiluted | Glycerol | 10% |
| KCl | 0.25 M | NP40 Detergent | 1% |
| | | EDTA | 5 mM |

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