DUROTEST® S

Product Code: P10 / P10A

Membrane test and reagents for semi-quantitative analysis of non-durum wheat adulteration in semolina.
For in vitro use only.

P10/V21/05.08.21

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RHÔNE LTD

Contents

Test Principle

The kit uses a monoclonal antibody which is specific for the protein friabilin which is present in non-durum wheats but not in durum wheat. Flour samples are extracted to release any friabilin protein. The extract is then absorbed onto the membrane, and the strip is saturated with a blocking agent. An enzyme labelled monoclonal antibody specific to the protein is then added. If non-durum wheat is present in the sample then a complex will be formed between the extracted friabilin protein and the enzyme labelled antibody. After washing the strip, a clear coloured staining reagent is added which forms a blue / purple coloured deposit in the presence of the friabilin /antibody enzyme complex. The degree of blue / purple colour with the sample is proportional to the percentage of non-durum wheat present. No colour developed indicates 100 % durum wheat (i.e. no adulteration).

The total assay time takes approximately 35 minutes to perform. The results can be compared to a 3 % non-durum wheat standard provided to give a semi-quantitative result.

Kit Components

- 20 membrane strips
- 1 vial containing 50 ml of extraction buffer at working strength
- 2 vials containing 50 ml of wash buffer at 10x concentration (green label)
- 20 incubation tubes containing 60 mg of blocking agent
- 80 microcentrifuge tubes
- 1 bottle containing 1.5 ml of antibody-HRP conjugate (red label) (P10A)
- 20 plastic pipettes
- 1 vial containing 33 ml of staining reagent A (blue label)
- 1 vial containing 33 ml of staining reagent B (blue label with white spot on lid)
- 2 g semolina standard sample 1: 100 % durum (grey label with black spot on lid)
- 2 g semolina standard sample 2: 3 % non-durum (orange label)

Note: The antibody-HRP conjugate (P10A) may be shipped separately due to the different storage conditions.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)

Hazards

Avoid skin contact with the following reagents:
- Extraction buffer: contains sodium dodecyl sulphate
- Staining reagents A and B: contains methanol / hydrogen peroxide

Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis. Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Storage & Shelf Life

The kit has an expiry of 12 months from date of manufacture if stored at 2 - 8 °C.

Note: Antibody-HRP conjugate (red label) (P10A) should be stored at -20 °C but will remain stable at temperatures above freezing for up to 4 days during transit.

Preparation of Wash Buffer (Green Label)

The wash buffer should be prepared fresh on the day of use and used within a 24 hour period.

1. Dilute a sufficient volume of wash buffer with water (1 : 10 (v/v)). For 1 strip dilute 5 ml of wash buffer with 45 ml of water.

2. Ensure that the wash buffer is at room temperature before use.

Sample Preparation

1. Weigh 100 mg (or 0.1 ml) of ground sample into one of the microcentrifuge tubes provided.

2. Mix each standard end over end and weigh 100 mg (or 0.1 ml) of each into additional microcentrifuge tubes provided.

3. Extract each sample by adding 0.5 ml of extraction buffer and either pipette up and down or invert to mix.

4. Leave for 3 minutes and then mix again.

5. Microcentrifuge the samples to clarify the supernatant (or allow solids to settle if preferred). If extracting larger samples, mix well, filter and use the filtrate in the assay.

6. Take a membrane strip and hold it by the clear plastic support, avoiding touching the white membrane surface. Place on a clean, flat surface with the absorbent membrane (dull side) uppermost.

7. Apply 5 µl of each supernatant centrally onto the white absorbent membrane with a clear margin between each. A maximum of 4 samples (e.g. 2 standards and 2 unknown samples) should be placed on each strip. Allow samples to absorb into the strips for 5 minutes.

8. Add 3 ml of diluted wash buffer to the graduation mark on the incubation tube. Replace the cap and mix well.

9. Remove the cap and insert the membrane strip (membrane end first) into the blocking solution.

10. Incubate the strip for 5 minutes, agitating the strip up and down in the solution to maximise blocking.

11. Remove the strip from the tube and place on a clean tissue.

12. Apply 2 drops of Antibody-HRP conjugate (red label) into the tube of blocking solution using the plastic pipette. Mix by inversion.

    Note: A fresh plastic pipette should be used for each sample.

13. Re-insert the strip into the solution and incubate for 20 minutes, occasionally agitating the strip up and down.

14. Remove the strip and rinse it with wash buffer, preferably using a wash bottle. Discard the contents of the tube and rinse it out with wash buffer. Replace the strip back in the tube and fill with wash buffer. Agitate the strip up and down in the buffer to remove any unbound antibody. Repeat this step a further 5 times to prevent high background colouration on development.

    Note: Washing of the strip must be thorough to prevent high background colouration on development.

15. Remove the strip and place on a clean surface.

16. Empty the incubation tube and invert it onto a paper towel to remove all traces of the buffer. Rinse and dry the cap. Add 1.5 ml of staining reagent A (blue label) and 1.5 ml of staining reagent B (blue label with white spot on lid) to the incubation tube. Mix the reagents thoroughly.

17. Insert the strip into the staining reagent and allow to incubate for 2 - 10 minutes in the dark. During this incubation check the strip frequently for the visible appearance of colour in standard 2 (3 % non-durum standard) and in any positive samples.

18. Rinse the strip in water to prevent excess colour development. Do not allow the colour to over develop or it may prevent accurate visual interpretation of the results. The colour will fade quickly as the strip dries so prompt estimation of unknown samples with the control standards is suggested.

Reading the Test Results

Standards	
100 % Durum Semolina Standard	3 % Non-Durum Semolina Standard
No colour development	Blue / purple colour

Samples	
Colouration Level	Adulteration Level
≥3 % non-durum standard	≥3 % common wheat
<3 % standard	<3 % non-durum standard
No colour development	No adulteration

Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical Support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to users of our products:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

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