UNIVERSITÀ DEGLI STUDI FIRENZE

Disclaimer

Il presente contenuto è stato prodotto per far fronte alle esigenze di didattica a distanza resasi necessarie per l'emergenza legata alla diffusione del virus COVID-19.

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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.

Campione incognito di sfarinato

Standard di farina 100% grano duro e 100% grano tenero forniti nel kit

Indica un anticorpo marcato con un enzima specifico HRP (perossidasi da rafano

Staining reagents indicano i substrati per l'enzima

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. Mix by inversion.
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.

1.  +3ml WASHING BUFFER (WB)
(l'agente bloccante si scioglie in WB)

2.

5min agitando ogni tanto

3.

 STRIP SU UN PEZZO DI CARTA


4.

20 min agitando ogni tanto

SVUOTARE IL TUBO E LAVARE LA STRIP con WB


5. + 3ml WB LAVARE PER 3-4 VOLTE

SVUOTARE IL TUBO


6. + 1.5 ml REAGENTE A + 1.5 ml REAGENTE B

2-10 min al buio (coprire il tubo)
controllare lo sviluppo del colore


LAVARE CON H2O E OSSERVARE LO SVILUPPO DI COLORE DELLA MACCHIA DEL CAMPIONE PER PARAGONE CON STD

Reading the Test Results

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

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

Sapete indicare a quale delle diverse classi dei test immuno-chimici che abbiamo affrontato a lezione appartiene questo protocollo?
A vostro giudizio, una volta definita la classe, quale sarà la presumibile relazione fra l'intensità osservata del colore e la concentrazione di antigene?