DURUM TEST ELISA ASSAY KIT

Enzyme immunoassay for the detection of gliadin from soft wheat species (*Triticum aestivum*) in flour and pasta

Reference: DURUM-381 (96 wells)

For *in vitro* diagnostic use only

Summary

Due to a specific spectrum of gliadins (the major reserve protein of cereals), pasta produced from durum wheat species (*Triticum durum*) flour preserves its shape well during cooking. According to most regulations, only durum wheat flour is used for manufacturing of high quality pasta. Flour obtained from soft wheat species (*Triticum aestivum*) is not suitable for pasta production, and if flour intended for pasta production contains admixture of flour obtained from soft wheat, this admixture may significantly impair the quality of the final product. Mixing of different wheat species may take place during harvesting, transportation, storage and milling of grains. Maximum acceptable content of soft wheat flour for pasta production in most common regulatory documents is set for 15%, though in some countries the regulations are more strict (down to 1%).

Intended use

The kit is intended for quantitative determination of soft wheat gliadin content in flour and pasta and allows 96 determinations or assaying of 40 samples in duplicates.

Principle of the test

This test is based on two-site sandwich enzyme immunoassay principle. An alcoholic extract of the tested pasta or flour is placed into non-coated microwells. Gliadin from the specimen adsorbs on the microwell surface. Unbound material is removed by washing procedure. Then specific monoclonal antibodies to *T. aestivum* gliadin (D-genome) are added to the wells with subsequent washing out of unbound material. Second polyclonal rabbit anti-mouse antibodies labeled with HRPO are then added. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of antigen in the specimen.

Kit contents

	Code	*Description*	Qty	Units	**Liquid** solution Color code
1	P381	Normal uncoated ELISA strips, 8x12 wells, breakable	1	pcs	
2	N003	Plate sealing tape	2	pcs	
3	C381x	Calibrator and control set, 0,1g each*	2	pcs	
4	S008Z	Washing solution concentrate **26x**, 22 ml	1	pcs	
5	A381Z	Solution of monoclonal antibodies to *T. aestivum* epitope, 11 ml	1	pcs	bright red
6	T381Z	Goat anti-mouse polyclonal antibodies conjugated to HRPO, 11 ml	1	pcs	bright blue
7	R055Z	TMB substrate solution, 14 ml	1	pcs	
8	R050Z	Stop solution, 14 ml	1	pcs	
9	K381IE	Instruction Durum Test EIA Assay Kit, English	1	pcs	
10	K381Q	QC data sheet Durum test EIA	1	pcs	
*	The set contains **5** calibrators containing 0, 1, 3, 10, 25% of soft wheat and 2 control samples.				

Materials required but not provided

- Distilled or deionized water;
- Ethylic alcohol

Necessary equipment

1. Microplate or Strip ELISA reader with 450 nm wavelength and OD measuring range 0-3.0.
2. Analytical balancing device with precision ≤ 1 mg.
3. Centrifuge with capability to spin 2 ml tubes at >= 200xg

HANDLING NOTES

1. Do not mix and/or use reagents from different lots within one run.
2. Replace caps on reagents immediately. Do not swap caps.
3. All kit components should be stored in the freezer (at +2 - +8°C). Do not freeze the kit or its components!

4. Do not use washing solutions containing sodium azide – even in trace quantities, it inhibits peroxidase, thus reducing color development.
5. Attention: during all incubations, please, seal the wells with adhesive tape, Do not allow drying of wells between assay steps.
6. It is recommended to assay all samples, calibrators and controls in duplicates.
7. Washing of wells may be made either manually or with automatic washing device. During each wash cycle, dispense 250 µl of Washing solution into each well. Soaking is not required. If washed manually, please, shake out the residual Washing solution from the wells by tapping on filter paper.
8. Please, measure OD in the wells within 15 minutes after addition of stop solution.

Assay procedure

Reagent preparation

All reagents (including the required number of strips) should be brought to Room Temperature (20-25°C) before use.

Sample preparation

*1	Grind a portion of dry pasta with a blender or a mortar. Continue grinding until a homogeneous powder is obtained. No grinding is necessary if flour is analyzed.
*2	Weight 100 ± 1 mg of unknown samples place them in tubes or vials. **Calibrators and controls are pre-weighed**. To extract gliadin from each of calibrator, control and unknown samples, add 1 ml of 70% ethanol to each sample.
*3	Incubate 60 min at 20-25°C on shaker platform.
*4	Centrifuge extracts 5 min at 200xg to eliminate particles. **Proceed to the Assay run or store the extracts up to 3 months in a freezer (below -15°C), in tightly closed vials or tubes.**

N.B.: For an optimal extraction of gliadin from calibrators, controls and sample, please take into consideration the following points:

- **Use a fresh 70% ethanol solution.**
- **Carry out the extraction horizontally (shake the tubes horizontally) and using an appropriate high shaking system to maximize the contact of the powder with the extraction solvent.**

Assay run:

*5	Put the desired number of microstrips into the frame; allocate two wells for each unknown sample and 16 wells for the calibrators and control samples.
*6	Dilute the extracts of calibrators, control and unknown samples 6-fold with 70% ethanol, e.g. add 200µl of extract to 1000µl of 70% ethanol; **diluted extracts are stable for up to 7 days in a freezer (below -15°C) in tightly closed glass vials or tubes. Do Not use plastic vessels to store diluted extracts!**
*7	Pipet 100µl of calibrator, control or unknown sample into the respective wells. Mix the contents carefully by moving the plate round and seal the wells with adhesive tape.
*8	Incubate 30 min at 20-25°C to adsorb gliadin from the samples onto the microwells surface.
*9	Prepare Washing solution by **26x** dilution of Washing solution concentrate (code S008Z) by distilled water. Diluted Washing solution is stable for 30 days at +2/8°C. Wash strips 3 times.
*10	Dispense 100µl of Monoclonal antibody to T. aestivum epitope into each well.
*11	Incubate 30 minutes at 20-25°C.
*12	Wash the strips 3 times.
*13	Dispense 100µl of conjugate into each well.
*14	Incubate 30 minutes at 20-25°C.
*15	Wash the strips 5 times.
*16	Pipet 100µl of Substrate into the wells.
*17	Incubate 15 minutes at 20-25°C.
*18	Pipet 100µl of Stop solution into the wells.
*19	Measure OD (optical density) at 450 nm.
*20	Set photometer blank on first calibrator.
*21	Use linear-log approximation to calculate results.

An example of calibration curve is given in the QC sheet.

Quality control

Control sample(s) should fall into the ranges shown in QC insert (see attached).

Expected values and normal range

Most of national regulatory acts require not more than 15% content of soft (*T. aestivum*) flour in ready pasta products.

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