Instructions for use

INTENDED USE
Phadebas® Honey Diastase Test is a method for the quantitative assay of α-amylase in all honey types.

PRINCIPLE OF THE PROCEDURE
Determination of the diastatic activity of honey utilising a photometric method in which a water-insoluble, cross-linked starch polymer carrying a blue dye is used as a substrate. The substrate is hydrolysed by α-amylase, yielding blue water-soluble fragments, determined photometrically at 620 nm. The absorbance of the solution is directly proportional to the diastatic activity of the sample. The method is based on that originally published by Siegenthaler (1) and modified by Bogdanov (2).

SPECIMEN COLLECTION AND HANDLING
- Avoid contamination with detergents, which may alter amylase activity, by using well rinsed glassware or disposable plastic equipment.
- Special care must be taken to avoid contamination with saliva or sweat, which contain large amounts of α-amylase.

MATERIAL PROVIDED, KIT CONTENTS
Phadebas® Honey Diastase Test (50) – one bottle of 50 tablets or Phadebas® Honey Diastase Test (500) – five bottles of 100 tablets. One tablet contains 45 mg blue starch.
Package insert.

SHELF LIFE AND STORAGE
Keep dry and store at controlled room temperature. After unsealing, store bottles closed, and retain desiccant. The expiry date is stated on the outer label.
N.B. Do not contaminate samples, tablets, or equipment with saliva or sweat.

DEFINITION
The unit of Diastase Activity, the Gothe unit, is defined as that amount of enzyme which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40°C under the conditions of test. Results are expressed in Gothe units (or Schade units) per gram of honey.

REAGENTS
Phadebas® Honey Diastase Test, Magle AB.
Sodium hydroxide (NaOH) solution, 0.5 M.
Acetate buffer (0.1M, pH 5.2): Dissolve 13.6 g of sodium acetate trihydrate in water. Adjust the pH of the solution to 5.2 with glacial acetic acid (1 - 2 ml) and dilute to 1L with distilled water.

EQUIPMENT
Photometer measuring at 620 ± 5 nm
Reagent mixer – vortex type
Thermostated water bath
Stop watch or timer
Centrifuge capable of producing 1500 g, or filtration equipment
Plastic centrifuge tubes
Volumetric flask
Tweezers
Automatic pipette or dispenser (5 ml)
Automatic pipette or dispenser (1 ml)
Disposable plastic tips

PROCEDURE
Preparation of test samples
If necessary, prepare the honey according to the section "Sampling" under the heading: INTRODUCTION AND GENERAL COMMENTS ON THE METHODS, in the Harmonised methods of the International Honey Commission, 2002.

Determination
Weigh 1.00 g of honey into a 100 ml volumetric flask, dissolve in the acetate buffer solution and fill to the mark. Complete the procedure within an hour. Transfer 5.0 ml of the solution to a test tube and place it in the water bath at 40°C for at least 5 minutes. Prepare a blank by placing a 5.0 ml aliquot of the acetate buffer in another test tube which is treated exactly as the sample solution. To both solutions add a Phadebas® tablet, using tweezers, and start the timer. Stir the solutions in the reagent mixer until the tablets disintegrate (ca. 10 seconds) and return them to the water bath. Terminate the reaction after exactly 30 minutes by adding 1 ml sodium hydroxide solution. Stir the mixture again in the reagent mixer for approximately 5 seconds. Immediately centrifuge at minimum 1500g for 5 minutes or filter the solutions through filter papers and measure the absorbance in 1 cm cuvettes at 620 nm using distilled water as reference. The absorbance of the blank is subtracted from that of the sample solution (ΔA_{620}). If the absorbance is higher than 1.0, dilute the sample with water. Take into consideration the dilution factor when calculating the results.

CALCULATION AND EXPRESSION OF RESULTS
The classical method for determination of diastase activity is the method of Schade (3, 4). A simultaneous measurement with the Phadebas® and the Schade method (3, 4) of 57 different commercial honey samples covering the range of diastase activity from 8 to 40 has been carried out. There was a very good correlation (r=0.987) between the two measurements. Linear regression of y (diastase number) against x (ΔA_{620}) yielded the following relation:

$$DN = 28.2 \times \Delta A_{620} + 2.64 \qquad (1)$$

where 28.2 and 2.64 are respectively the slope and the intercept of the best straight line obtained by linear regression of ΔA_{620} (x axis) on DN (y axis).

If (1) yields a diastase activity below 8 DN, equation (2) below gives a better correlation and should be used. For low diastase values (0-6 DN), a very good correlation (R2 = 0,927) was found and linear regression of y (diastase number) against x (ΔA_{620}) yielded the following relation

$$DN = 35.2 \times \Delta A_{620} - 0.46 \qquad (2)$$

where 35.2 and 0.46 are respectively the slope and the intercept of the best straight line obtained by linear regression of ΔA_{620} (x axis) on DN (y axis). Equation 2 should be used if equation (1) yields a diastase activity below 8 DN.

PRECISION
a) Precision data determined in a Swiss data trial (5):
1. Three different types of honey were tested by three laboratories. The maximum deviation (range) of the diastatic activity determined with tablets of the same batch, between the laboratories, was found to be 3.7 %

2. The standard deviation of the diastatic activity determined with tablets of two different batches with the same honey, within one laboratory, was 3.7 % (for n=24, n being the number of analyses per batch).

3. The weight range, for a sample of 20 tablets, was found to be 5 %, with a standard deviation of 2 %.

b) Interlaboratory trial with the Phadebas® method
An interlaboratory trial on the Phadebas® method was coordinated by the International Honey Commission in 1992 with 14 EC and 21 Swiss laboratories, and carried out using honeys with A_{620} values varying from 0.31 to 1.29 (6). The batches of Phadebas® reagent were not specified.

The following r and R values were obtained:

A-620	0.212	0.314	0.414	0.588	0.704	0.705	0.734	0.970	1.294
r	0.034	0.032	0.032	0.042	0.049	0.043	0.050	0.065	0.060
R	0.107	0.134	0.161	0.202	0.273	0.311	0.250	0.336	0.428

where A-620 is the absorbance value.

The repeatability and reproducibility have been calculated from the results on nine types of honey analysed by all laboratories collaborating in the study. The following correlation equations were calculated from this data:

$$r = 0.02 + 0.03 \times A_{620}$$

$$R = 0.04 + 0.32 \times A_{620}$$

REFERENCES

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2. S. Bogdanov, Honigdiastase, Gegenüberstellung verschiedener Bestimmungsmethoden, Mitt. Gebiete Lebensmitt.Hyg. 75, 214-220 (1984).
3. J.E.Schade, G.L.Marsh and J.E.Eckert: Diastase activity and hydroxymethylfurfural in honey and their usefulness in detecting heat adulteration. Food Research 23, 446-463 (1958).
4. DIN-NORM 10750 Bestimmung der Diastase-Aktivität. (1990).
5. Bestimmung der Amylactivität (nach Phadebas), Schweizer Lebensmittelbuch Kapitel 23 A: Honig, EDMZ, Bern, (1995).
6. S.Bogdanov and P. Lischer, Interlaboratory trial of the International Honey Commission: Phadebas and Schade Diastase determination methods, Humidity by refractometry and Invertase activity: Report for the participants (1993).

WARRANTY

Any change or modification in the procedure not recommended by Magle AB may affect the results, in which event Magle AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.
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OD 620nm	DN	OD 620nm	DN	OD 620nm	DN	OD 620nm	DN	OD 620nm	DN	OD 620nm	DN
0,040	0,9	0,200	8,3	0,360	12,8	0,540	17,9	0,860	26,9	1,180	35,9
0,045	1,1	0,205	8,4	0,365	12,9	0,550	18,2	0,870	27,2	1,190	36,2
0,050	1,3	0,210	8,6	0,370	13,1	0,560	18,4	0,880	27,5	1,200	36,5
0,055	1,5	0,215	8,7	0,375	13,2	0,570	18,7	0,890	27,7	1,220	37,0
0,060	1,7	0,220	8,8	0,380	13,4	0,580	19,0	0,900	28,0	1,240	37,6
0,065	1,8	0,225	9,0	0,385	13,5	0,590	19,3	0,910	28,3	1,260	38,2
0,070	2,0	0,230	9,1	0,390	13,6	0,600	19,6	0,920	28,6	1,280	38,7
0,075	2,2	0,235	9,3	0,395	13,8	0,610	19,8	0,930	28,9	1,300	39,3
0,080	2,4	0,240	9,4	0,400	13,9	0,620	20,1	0,940	29,1	1,320	39,9
0,085	2,5	0,245	9,5	0,405	14,1	0,630	20,4	0,950	29,4	1,340	40,4
0,090	2,7	0,250	9,7	0,410	14,2	0,640	20,7	0,960	29,7	1,360	41,0
0,095	2,9	0,255	9,8	0,415	14,3	0,650	21,0	0,970	30,0	1,380	41,6
0,100	3,1	0,260	10,0	0,420	14,5	0,660	21,3	0,980	30,3	1,400	42,1
0,105	3,2	0,265	10,1	0,425	14,6	0,670	21,5	0,990	30,6	1,420	42,7
0,110	3,4	0,270	10,3	0,430	14,8	0,680	21,8	1,000	30,8	1,440	43,2
0,115	3,6	0,275	10,4	0,435	14,9	0,690	22,1	1,010	31,1	1,460	43,8
0,120	3,8	0,280	10,5	0,440	15,0	0,700	22,4	1,020	31,4	1,480	44,4
0,125	3,9	0,285	10,7	0,445	15,2	0,710	22,7	1,030	31,7	1,500	44,9
0,130	4,1	0,290	10,8	0,450	15,3	0,720	22,9	1,040	32,0	1,520	45,5
0,135	4,3	0,295	11,0	0,455	15,5	0,730	23,2	1,050	32,3	1,540	46,1
0,140	4,5	0,300	11,1	0,460	15,6	0,740	23,5	1,060	32,5	1,560	46,6
0,145	4,6	0,305	11,2	0,465	15,8	0,750	23,8	1,070	32,8	1,580	47,2
0,150	4,8	0,310	11,4	0,470	15,9	0,760	24,1	1,080	33,1	1,600	47,8
0,155	5,0	0,315	11,5	0,475	16,0	0,770	24,4	1,090	33,4	1,620	48,3
0,160	5,2	0,320	11,7	0,480	16,2	0,780	24,6	1,100	33,7	1,640	48,9
0,165	5,3	0,325	11,8	0,485	16,3	0,790	24,9	1,110	33,9	1,660	49,5
0,170	5,5	0,330	11,9	0,490	16,5	0,800	25,2	1,120	34,2	1,680	50,0
0,175	5,7	0,335	12,1	0,495	16,6	0,810	25,5	1,130	34,5	1,700	50,6
0,180	5,9	0,340	12,2	0,500	16,7	0,820	25,8	1,140	34,8	1,720	51,1
0,185	6,1	0,345	12,4	0,510	17,0	0,830	26,0	1,150	35,1	1,740	51,7
0,190	8,0	0,350	12,5	0,520	17,3	0,840	26,3	1,160	35,4	1,760	52,3
0,195	8,1	0,355	12,7	0,530	17,6	0,850	26,6	1,170	35,6	1,780	52,8

Phadebas®

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