BZERO STREPTO

Enzyme immunoassay for the detection of streptomycin (Cat.nr. HU0050024)

BZERO **STREPTO** is a kit prepared for a competitive immunoenzymatic assay for the quantitative analysis of streptomycin.
The kit contains the procedure and the materials sufficient for 48 determinations.
For result evaluation a microtiter plate or a strip photometer is required (manual or automatic ELISA reader).

Type of samples that can be analyzed (matrices)
Honey, royal jelly, muscle, milk.
For analysis of milk samples, contact the technical assistance (Support.ET.trieste@eurofins.com).

Sample preparation
Honey: dilution and filtration.
Honey (high sensitivity procedure): dilution, incubation, further dilution.
Royal jelly: acid extraction, centrifugation, pH adjustment, centrifugation, dilution.
Muscle: grinding, acidification, homogenization, acid extraction, cooling, centrifugation, dilution.

Assay time: 60 minutes (sample preparation not included).

Detection limit
Honey, royal jelly: 2 ppb.
Honey (high sensitivity procedure): 0.75 ppb
Muscle: 5 ppb.

Specificity	
Analyte	**Cross-reactivity (%)**
Streptomycin	100
Dihydrostreptomycin	103±5

1. TEST PRINCIPLE

The assay is performed in plastic microwells which have been coated with anti-streptomycin antibodies.
Standard, samples and enzyme conjugate are added to the microwells
During the first incubation, free - streptomycin molecules in the samples and the enzyme conjugate, compete for the anti- streptomycin antibody binding site. Any unbound substance is then removed in a washing step.
The enzyme conjugate-antibody binding is then detected by adding the colourless substrate/chromogen solution, which during the second incubation is converted by the enzyme into a blue reaction product. The addition of the stop reagent leads to a colour change from blue to yellow. The absorbance is measured with a microplate reader at 450 nm. The colour development is inversely proportional to the streptomycin concentration in the standard/sample.

2. PROVIDED REAGENTS

Microtiter plate: 48 wells (6 strips x 8 wells), coated with anti-streptomycin antibodies
As the strips are breakable, the wells can be used individually. For this purpose, it is sufficient to break the joint.
Std 0: 1 vial containing 1.5 ml of streptomycin standard of 0 ng/ml. **ATTENTION: Only the standard zero is provided. B/B_0 values of calibration curve (0.1-20 ng/ml) are reported in the kit certificate of analysis.**
Enzyme Conjugate: 1 bottle containing 5 ml.
Dilution buffer 20x: 1 bottle containing 50 ml.
Washing-buffer 10x: 1 bottle containing 50 ml.
Developing solution: 1 bottle containing 8 ml.
Stop solution: 1 glass bottle containing 6 ml. White cap.

3. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled water
- 0.1 M H_3PO_4 (royal jelly).
- 1 M NaOH (royal jelly, muscle)
- 3% Trichloracetic acid (muscle)
- 50 mM carbonate buffer pH 9.6 containing 0.5 mM EDTA (honey, high sensitivity). For preparation, see chapter 7.

Equipment:
- Balance (honey, royal jelly, muscle).
- Vortex (optional) (honey, royal jelly).
- Shaker (royal jelly).
- Tissue homogenizer (muscle)
- Rotating wheel (muscle).
- Incubator or water bath (honey).
- Centrifuge (royal jelly).
- Filter paper (Whatman n. 1) (honey).
- 20-200 µl, micropipettes, tips.
- 100-1000 µl, micropipettes, tips.
- 50-250 µl, multichannel micropipette, tips.
- Microplate reader, filter 450 nm

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4. WARNING AND PRECAUTIONS FOR THE USERS

- The test is for *in vitro* diagnostic use only.
- Some reagents contain solutions that may be identified as dangerous substance by the Regulation (EC) *N° 1272/2008.* Please refer to *Material Safety Data Sheet* available on both the Eurofins Technologies and Eurofins Tecna (*tecna.eurofins-technologies.com)* web site.
- Handle the reagents with caution, avoiding contact with skin, eyes and mucous membranes.

5. HANDLING AND STORAGE INSTRUCTIONS

- Store the kit at +2/+8 °C and never freeze.
- **Bring all reagents to room temperature before use (at least 1 hour). ATTENTION:** Do not unseal the microplate until it reaches the room temperature.
- Reseal the unused strips of the microtiter plate in the bag together with the desiccant bag provided.
- Return all reagents to +2/+8 °C immediately after use.
- Do not use components after the expiration date.
- Do not intermix components from different kit lots.
- Do not use photocopies of the instruction booklet. Follow the original instruction booklet that is included with the kit.
- Do not change the assay procedure, in particular:
  - do not prolong the incubation times,
  - do not incubate the plate at temperatures higher than 25 °C,
  - do not shake the plate during the incubations.
- Use accurate and precise micropipettes with suitable tips for dispensing.
- Once started, complete all the steps without interruption.
- The reproducibility of ELISA results depends largely upon the efficiency and uniformity of microwells washing; always keep to the described procedure.
- Use a single disposable tip for each standard solution and sample to avoid cross-contamination.
- Do not allow tips to contact the liquid already in the microwells.
- Avoid exposure to direct light during all incubations. It is recommended to cover the microtiter plate without using plate sealers.
- **ATTENTION: We warn to handle the reagents and the sample extracts with care in order to avoid cross-contaminations, mainly in case of spiking experiments.**

6. SAMPLE PREPARATION

6.1 Honey

ATTENTION: It is suggested to use only plastic tubes, do not use glass

1) Weigh 1 g of homogeneous honey sample.

ATTENTION: If crystals are present, warm the sample overnight (do not exceed 45°C) and then mix properly before weighing the sample**.**

2) Add 19 ml of dilution buffer 1x
3) Shake or vortex until honey is completely dissolved.
4) Filter with Whatman 1.
5) Dilution factor is 20.
   It is suggested to test the samples immediately after preparation.

Alternative procedure:

1) Weigh 1 g of homogeneous honey sample

ATTENTION: If crystals are present, warm the sample overnight (do not exceed 45°C) and then mix properly before weighing the sample**.**

2) Add 9 ml of dilution buffer 1x.
3) Shake manually or vortex until honey is completely dissolved.
4) Filter with Whatman 1.
5) Dilute the filtrate 1:2 (1+1) with dilution buffer 1x.
6) Dilution factor is 20.

It is suggested to test the samples immediately after preparation.

6.2 Honey (high sensitivity procedure)

ATTENTION: It is suggested to use only plastic tubes, do not use glass

1) Weigh 1 g of homogeneous honey sample.

ATTENTION: If crystals are present, warm the sample overnight (do not exceed 45°C) and then mix properly before weighing the sample**.**

2) Add 2 ml of 50 mM carbonate buffer pH 9.6 containing 0.5 mM EDTA.
3) Shake or vortex until honey is completely dissolved.
4) Incubate at 40°C for 10 minutes.
5) Mix well and dilute 1:2.5 with distilled water (for example: 100 µl of sample + 150 µl of water).
6) Dilution factor is 7.5.

It is suggested to test the samples immediately after preparation.

It is recommended to not analyse more than 10 sample for each analytical session.

6.3 Royal jelly

1) Weigh 1 g of homogeneous royal jelly sample
2) Add 2 ml of 0.1 M H_3PO_4.
3) Shake manually or vortex until the sample is completely dissolved.
4) Shake samples for 10 minutes.
5) Centrifuge at 2200 g for 5 minutes.

6) Transfer the supernatant in a new vial and add 450 µl of 1M NaOH:
7) Verify that the pH is between 7 and 9.
8) Centrifuge at 2200 g for 5 minutes.
9) Dilute 50 µl of supernatant with 350 µl of dilution buffer 1x.
10) Dilution factor is 20.

6.4 Muscle

1) Remove the fatty and fibrous parts of the tissue.
2) Grind at least 100 g of sample.
3) Weight 1 g of the sample.
4) Add 4 ml of 3% trichloracetic acid.
5) Homogenize for 1 minute at high speed
6) Extract for 30 minutes on a rotating wheel.
7) Cool the samples on ice or at +2/+8°C.
8) Centrifuge at 2000g for 10 minutes, preferably at +2/+8°C.
9) Dilute 200 µl of the clear supernatant with 1800 µl of dilution buffer 1x.
   ATTENTION: If the supernatant is not clear centrifuge it once again or filtrate)
10) Adjust carefully the pH to 7,4 with 1M NaOH.
11) The dilution factor is 50.

7. WORKING SOLUTIONS PREPARATION

Std 0: ready to use.
Enzyme Conjugate: ready to use.
Dilution buffer: dilute it 1:20 (1+19) with distilled water.
ATTENTION: In presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
Washing buffer: dilute the concentrated buffer **1:10** (1+9) with distilled water. **ATTENTION:** In presence of crystals, bring the solution at room temperature and stir in order to solve them completely.
The diluted washing buffer is stable at room temperature for 24 hours and at +2/+8°C for two weeks.
Developing solution: ready to use; this solution is light sensitive: keep away from direct light.
Stop solution: ready to use. **ATTENTION:** It contains 1M sulphuric acid. Handle with care; in case of contact flush immediately with plenty of water.
Honey dilution buffer (high sensitivity procedure). For 50 ml of 50 mM carbonate buffer pH 9.6 containing 0.5 mM EDTA: mix 5 ml of 500 mM carbonate buffer pH 9.6 with 0.5 ml of 50 mM EDTA pH 8 and add distilled water up to 50 ml.

8. ASSAY PROCEDURE

1) Predispose an assay layout, recording the standard 0 and samples positions, taking into account that all have to be run in single wells.
2) First incubation
   - Add 50 µl of standard 0 / samples into the corresponding wells.
   - Using the multichannel pipet, add 50 µl of enzyme conjugate in each well.
   - Shake the plate gently with rotatory motion for few seconds.
   - Incubate 30 minutes at room temperature.
   - Do not prolong the first incubation time and do not use automatic shakers.
3) Washing
   - Pour the liquid out from the wells.
   - Fill completely all the wells with whashing buffer 1x using a squeeze bottle. Pour the liquid out from the wells.
   - Repeat the washing sequence three (3) times.
   - Remove the remaining droplets by tapping the microplate upside down vigorously against absorbent paper.

Do not allow the wells to dry out

4) Developing
   - Using the multichannel pipet, add 100 µl of developing solution to each well.
   - Mix thoroughly with rotatory motion for few seconds.
   - Incubate for 30 minutes at room temperature. Protect from direct light.
5) Using a multichannel pipet, add 50 µl of stop solution to each well and mix thoroughly with rotatory motion for few seconds.
6) Measure the absorbance at 450 nm.
7) Read within 60 minutes.

9. CALCULATION OF RESULTS

- Divide the absorbance value of each sample by the absorbance of the standard 0 (B_0) and multiply by 100; the standard 0 is thus made equal to 100% and all the other absorbance values are expressed as percentages:

$$\frac{\text{Sample absorbance}}{\text{Standard 0 } (B_0) \text{ absorbance}} \times 100 = \frac{B}{B_0}(\%)$$

- Enter the B/B_0 values provided in the certificate of analysis for each standard (0,1; 0,25; 1; 5; 20 ng/ml) in a semi-logarithmic system of coordinates and draw the standard curve.
- Interpolate the B/B_0 value of each sample to the corresponding concentration (ng/ml) from the calibration curve. For dilution applications multiply this concentration for the dilution factor, as reported in chapter 6.

Please note: to elaborate the ELISA results the "point to point" calibration curve is suggested. Excel spreadsheet are available on Eurofins Tecna website tecna.eurofins-technologies.com and can be downloaded directly from the bottom of the product page.

10. EVALUATION OF RESULTS

After processing the results, it is necessary to verify the assay performance. The verification is performed by comparison of obtained data with those given in kit specifications (see chapter 11).
If the values are outside the specifications given, then the results of the test are not assured, therefore the streptomycin concentration levels in the samples may not be valid.
In these cases, it is advised to check the expiry date of the kit, the wavelength at which the reading was performed, as well as the procedure followed.
If operation errors are not identified as cause, contact our technical assistance.
In order to avoid false positive results, it is necessary to adopt a decision limit (CCα). It is suggested to adopt an internal CCα. For further information, contact the technical assistance

WARNING: Kit replacement will only be possible in case of return. The kit must be stored in its integral version at +2/+8°C.

11. KIT SPECIFICATIONS

11.1 Assay specification

Description	Specifications
B_0 absorbance	$\geq$ 0.7 OD_{450nm}

11.2 Assay performances

Detection Capability (CCβ) was calculated as requested by of EU Decision 657/2002.

Detection capability	
Sample	**CCβ**
Muscle	20 ppb
Honey	5 ppb
Honey (high sensitivity)	1 ppb

12. LIABILITY

Samples evaluated as positive using the kit have to be re-tested with a confirmation method.
Eurofins Technologies Hungary shall not be liable for any damages to the customer caused by the improper use of the kit and for any action undertaken as a consequence of results.
Eurofins Technologies Hungary shall not be liable for the unsafe use of the kit out of the current European safety regulations.

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