

I'screen STREPTO

Enzyme immunoassay for the detection of streptomycin

(Cat.nr. HU0050013)

I'screen 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 96 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 (Export.ET.Trieste@eurofins.com).

This ELISA kit is not intended to be used for testing any kind of human samples.

Sample preparation

Honey: dilution and filtration.

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.

Muscle: 5 ppb.

Analyte	Specificity	
	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.

Standards, 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 standards/sample.

2. PROVIDED REAGENTS

Microtiter plate: 96 wells (12 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.

Streptomycin Std: 6 plastic vials containing 1.5 ml of streptomycin in the following concentrations: 0 ng/ml; 0.1 ng/ml; 0.25 ng/ml; 1 ng/ml; 5 ng/ml; 20 ng/ml.

Enzyme conjugate: 1 plastic bottle containing 8 ml.

Dilution buffer 20x: 1 bottle containing 50 ml.

Washing-buffer 10x: 1 bottle containing 50 ml.

Developing solution: 1 bottle containing 14 ml.

Stop solution: 1 glass bottle containing 8 ml. White cap.

3. MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled water
- 0.1 M H₃PO₄ (royal jelly).
- 1 M NaOH (royal jelly, muscle).
- 3% Trichloroacetic acid (muscle).

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 450nm.

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 Royal jelly

- 1) Weigh 1 g of homogeneous royal jelly sample
- 2) Add 2 ml of 0.1 M H₃PO₄.

- 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.

6.3 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% trichloroacetic 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

Streptomycin Std: 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.

8. ASSAY PROCEDURE

- 1) Predispose an assay layout, recording the standard and samples positions, taking into account that all have to be run in duplicate. **ATTENTION:** It is suggested to carry out no more than 48 determinations in each assay (standards included).
- 2) First incubation
 - Add 50 µl of standard/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 washing 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

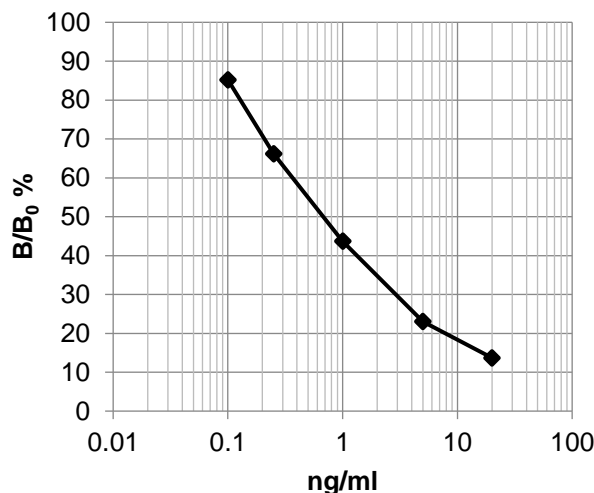
- Divide the absorbance value of each sample by the absorbance of the standard 0 (B₀) and multiply by 100; the standard 0 is thus made equal to 100% and all the other absorbance values are expressed as percentages:

$$\frac{\text{Standard (or sample) absorbance}}{\text{Standard 0 (B}_0\text{) absorbance}} \times 100 = \frac{B}{B_0} (\%)$$

- Enter the B/B₀ 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/B₀ 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: for results calculation it is suggest to use the "point to point" curve The "spline" curve or the "4 Parameters" can also be used. Do not use the linear regression. For the "point to point" curve, 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. EXAMPLE OF STANDARD CURVE



11. 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 12).

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 (CC_α). It is suggested to adopt an internal CC_α. 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.

12. KIT SPECIFICATIONS

12.1 Assay specifications

Mean B ₀ absorbance	≥ 0.7 OD _{450nm}
B/B ₀ 50%	0.2 – 1.5 ng/ml
Std. duplicates mean C.V. (%)	≤ 6

12.2 Assay performance

The kit performances hereby presented are from an in-house validation; Detection Capability (CC_β) was calculated as requested by of EU Decision 657/2002. A Performance Data Sheet (PDS) with more detailed information is available upon request.

Detection capability or CC β	
Sample	CC β
Muscle	20 ppb
Honey	5 ppb
Royal jelly	5 ppb

Accuracy	
Sample	Recovery %
Bovine muscle (spiking at 500 ppb of streptomycin)	89 \pm 22
Suine muscle (spiking at 500 ppb of streptomycin)	84 \pm 4
Honey (naturally contaminated samples)	112 \pm 28
Royal jelly (spiking at 10 ppb of streptomycin)	114 \pm 14

The results were obtained by means of a "4 parameters" elaboration of the calibration curve.

13. 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.

14. LITERATURE

Diana F., Rosar G., Persic L., Paleologo M. I'screen STREPTO: a new enzyme immunoassay for the quantitative determination of streptomycin and dihydrostreptomycin in honey. Euroresidue VII: Conference on Residues of Veterinary Drugs in Food, 14-16 May 2012, Egmond aan Zee, The Netherlands. Proceedings, 461-466.