Tetrasensor

Assay for tetracycline residues in animal tissues.

Tissue Tetrasensor Kits.

Contact.

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Introduction.

Tetrasensor is a receptor-based assay for rapid determination of the amount of tetracycline present in a food sample. The screening test allows to detect all tetracycline molecules (tetracycline, oxytetracycline, doxycycline and chlortetracycline) at 20 ppb for TM00629 (25 assays) and TM00630 (100 assays), while TM00356 (25 assays) and TM00628 (100 assays) are the Universal Tissue kits with sensitivity at 100 ppb (MRL).
The test can be performed on muscle tissues that are originated from many animal species like Beef, Pork, Chicken, … Moreover, presence of tetracycline in Seafood, Eggs, Fish and urine can also be determined with Tetrasensor.
For Milk refer to product TK00543 or TK00544 and for Honey refer to TH00616 or TH00624.

Reaction mechanism.

Tetrasensor is a competitive test that exploits the activity of a receptor for the recognition of tetracycline molecules present in the sample. The test requires the use of two elements provided in the kit. The first element is a reagent containing a certain amount of labelled receptor and the second is a dipstick consisting of a set of membrane where two capture lines are printed in green. When there is a liquid format of your tissue sample, the sample supernatant is added together with the receptor and the dipstick. While starting to run vertically on the strip, the receptor binds tetracycline molecules present in the sample. When the liquid passes through the green capture lines, red colour appears. The first line captures the remaining active receptor and the second line takes a certain amount of the excess of reagent that has passed through the first line. This second upper line serves as a control line and becomes visible in all cases.
The amount of receptor introduced in the vial determines the limit of detection. Therefore in TM00356 we have set this amount to get a negative answer below the minimum of all tetracycline MRL values set at 100ppb for muscle tissues. In consequence, basic versions (TM00356 & TM00628) gives you 100% of NEGATIVE response at 50ppb and 100% of POSITIVE response at 100ppb. To be around the MRL for kidney, which is 6 times higher than for muscle, the extract must be diluted 6 times more. For eggs, place liquid dilution directly into the reagent vial without additional dilution (see detail).

Composition of kits.

TM00356, MRL, 25 Assays, (basic version) contains:
- 25 reagent vials containing the freeze-dried labelled receptor.
- 25 dipsticks placed in plastic bottle with desiccant.
- 25 plastic pouches for tissue sample preparation.
- 1 bottle of 100ml "Tissue Buffer 10X".
- 25 disposable pipettes.
- 25 disposable tips (200µl tips).
- 25 Eppendorfs of ready-to-use "Dilution Buffer 1X".
- 25 Empty tubes.
- 1 graduated measuring cylinder.
- 1 minipet (200 µl).
- 1 information notice.

TM00628, MRL, 100 Assays, contains:
- 100 reagent vials containing the freeze-dried labelled receptor.
- 4 x 25 dipsticks placed in plastic bottle with desiccant.
- 1 bottle containing 100ml "Tissue Buffer 40X".
- 1 information notice.

TM00629, limit at 20ppb, 25 Assays, contains:
- 25 reagent vials containing the freeze-dried labelled receptor.
- 25 dipsticks placed in 1 plastic bottle with desiccant.
- 25 plastic pouches for tissue sample preparation.
- 1 bottle containing 50 ml of "Tissue Buffer 20X".
- 1 bottle containing 15 ml of "Urine Buffer 1X".
- 1 information notice.

TM00630, limit at 20ppb, 100 Assays, contains:
- 100 reagent vials containing the freeze-dried labelled receptor.
- 4 x 25 dipsticks placed in 4 plastic bottle with desiccant.
- 1 bottle containing 175ml of "Tissue Buffer 20X".
- 1 bottle containing 60 ml of "Urine Buffer 1X".
- 1 information notice.

Remarks for storage conditions.

We recommend storing your Kit at 4°C. upon arrival.
Let products reach room temperature before opening and avoid exposure to moisture.
The best temperature to perform one test is 25°C ± 5°C.

Material required* for Solid tissue sample preparation

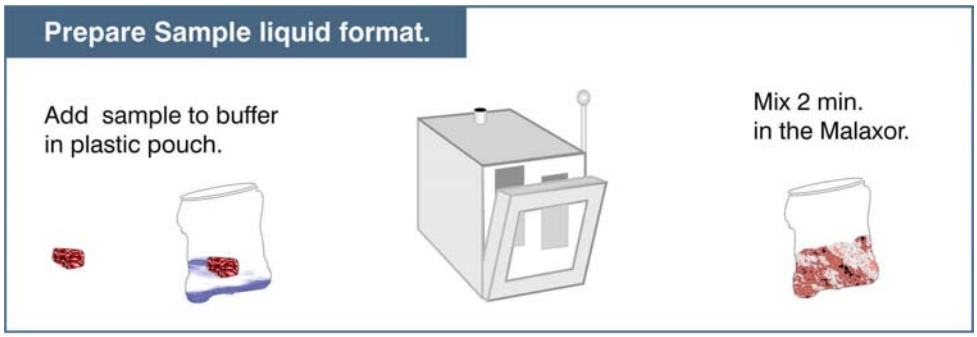
- Balance.
- Malaxor or Masticator.
- Plastic pouches for Malaxor (included in TM00356 and TM00629).
- Eppendorf Centrifuge.
- Eppendorf tube (included in TM00356).
- Distilled or pure water.
- Graduated measuring cylinder (included in TM00356).
- Tips and pipettes (included in TM00356).

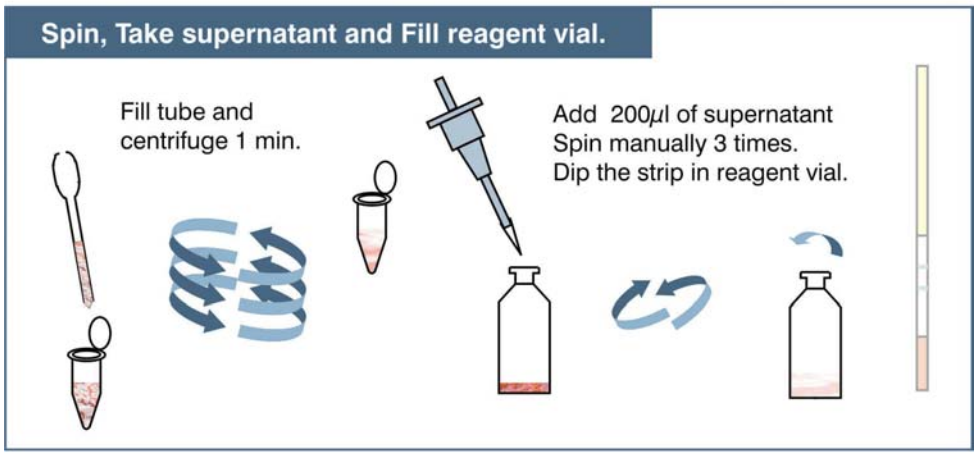
Material required* for Optical measurement & datalogging

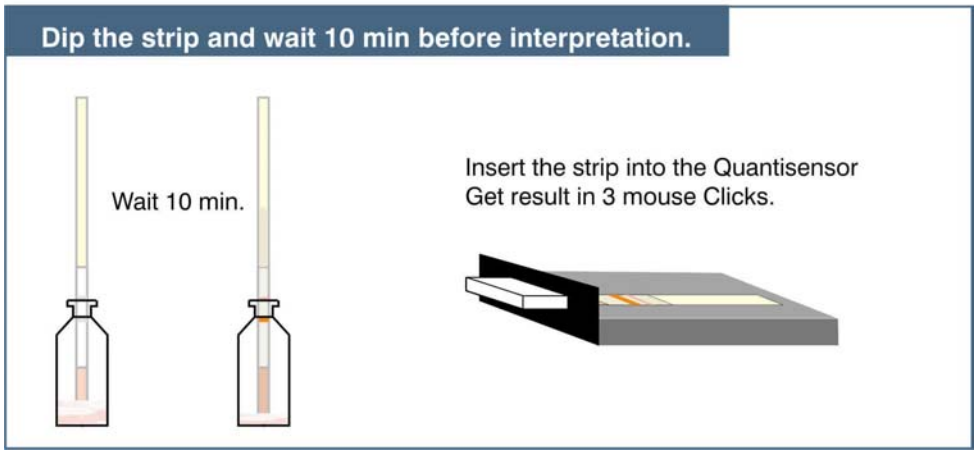
- Quantisensor dipstick reader.
- Personal Computer and printer.

** Required material can be available from Unisensor; Optical measurement is an OPTION.*

The Procedure Scheme.







- **Add 10 g. of tissue and 30 ml of Buffer(*) into one plastic pouch.**
- **Mix continuously over 2 minutes in the stomacher.**
- **Take 1 ml and Centrifuge 1 minute.**
- **Fill reagent vial with 200 µl of supernatant (or dilution).**
- **Dip one dipstick into the vial.**
- **Incubate over 10 minutes at RT.**
- **Interpret by comparing the coloured lines you get on the strip.**
- **Use Quantisensor for optical measurement and results storage.**

Advantages of the method.

- The system allows you to make a test in less than 15 minutes starting with a solid sample tissue.
- Ready to use individual vials and dipsticks mean no risk of cross contamination.
- Individual freeze-dried vial guarantied long storage without lost of activity.
- Easy print-out and data storage with the Quantisensor optional system.

General remarks.

The kit TM00356 is the basic version. Reactants provided in the kit allows you to adapt, with very ease, the sensitivity of the test to the required MRL in application with respect to the type of tissue sample. If you strictly follow the standard procedure with TM00356, the sensitivity will be automatically set at 100 ppb of tetracycline whatever the sample tissue. This can be done in routine analysis for instance or if the sample is muscle tissue (bovine; pork; fish; …). If you want to set the sensitivity in relation to the appropriate MRL (Kidney: 600 ppb; lever: 300 ppb; egg: 200 ppb) follow the instruction in "Type of Tissue - Remarks for TM00356" and make the appropriate dilution with the buffer provided in the kit.

The Standard Procedure.

Whatever the sample is the following Procedure set the limit of the detection as mentioned on top of the kit.

1. Wash and dry hands thoroughly and take the box out of the fridge.

   Extract antibiotic molecules by preparing a liquid format of your tissue to analyse.
   *For **Seafood** samples use the seafood body cleared from the shell.*
   Before use, make a ready to use buffer and proceed with dilution as mentioned on the buffer bottle label. Use pure water and label your bottle as "Tissue Buffer 1X". You need about 30 ml per 10g of solid sample. For liquid format preparation proceed as follow:

2. Weight approximately ten (10) grams of tissue.
3. Take one new plastic pouch and introduce the weighted tissue.
4. Use one graduated measuring cylinder to take the appropriate quantity of "tissue buffer 1X" you need in respect to the precise weight of tissue you have taken.
   Help you with the annexed Tab n°1.
5. Add the corresponding volume of buffer into the plastic pouch.
6. Place the pouch in the malaxor and run the system continuously over two (2) minutes in maximum speed.
7. With the help of one disposable pipette, fill one fresh centrifuge tube with homogenate.
8. Centrifuge the homogenate one (1) minute in a table centrifuge (10.000 rpm) to separate supernatant from solid material.
9. Take out one individual reagent vial and remove rubber cap.
10. Place a new disposable tip on your 200µl pipette.
11. Open the dipsticks bottle, take one dipstick and close the bottle. Print on each dipstick the appropriate sample identification number.
12. Take the tube from the centrifuge and transfer 200 µl of solution into the reagent vial.
13. Spin manually 3 times to dissolve the dried pellet of reagent (about 15 to 20 seconds).
14. Immediately after dissolving the pellet, dip one dipstick into the vial in a way to submerge the bottom edge of the dipstick (arrows downwards) and start an incubation over ten (10) minutes at room temperature.
15. After 10 minutes, take out the dipstick of the vial. You get one or two coloured red lines printed on your strip that must be interpreted or quantified immediately (see next page).
16. If you do not intent to run a new test, please close firmly the dipsticks bottle and replace the Tetrasensor box containing reagent vials and dipsticks into the fridge.

Tab n°1: Correspondence of volume of tissue buffer 1X versus weight of tissue (*).

Tissue Weight	Buffer Volume	Tissue Weight	Buffer Volume	Tissue Weight	Buffer Volume	Tissue Weight	Buffer Volume
6,50 g	19,50 ml	8,00 g	24,40 ml	9,50 g	28,50 ml	11,00 g	33,00 ml
6,75 g	20,25 ml	8,25 g	24,75 ml	9,75 g	29,25 ml	11,25 g	33,75 ml
7,00 g	21,00 ml	8,50 g	25,50 ml	10,00 g	30,00 ml	11,50 g	34,50 ml
7,25 g	21,75 ml	8,75 g	26,25 ml	10,25 g	30,75 ml	11,75 g	35,25 ml
7,50 g	22,50 ml	9,00 g	27,00 ml	10,50 g	31,50 ml	12,00 g	36,00 ml
7,75 g	23,25 ml	9,25 g	27,75 ml	10,75 g	32,25 ml	12,25 g	36,75 ml

(*) We recommend adding the appropriate volume of buffer in respect to the weight of tissue to set the tetracycline screening test at the indicated sensitivity.

Type of tissue - Remarks for TM00356.

Whatever the sample is, the Standard Procedure set the limit of the detection at 100 ppb for tetracycline (Muscle MRL). If you want to adjust your specific tissue preparation to the appropriate MRL limit, please adapt dilutions as follow.

- *For **egg** samples only, escape step 6 to 8 because you start with a liquid sample and proceed as follow:*
  In step 5, mix gently to avoid foaming and continue in step 9 to 11 like in the standard procedure. In step 12 take 200 µl of this mix to fill the opened reagent vial. Follow instructions in step 13.
- *For **kidney** samples tissue only, dilute 6 times more the supernatant to set the detection sensitivity at the appropriate kidney MRL (600ppb) and proceed as follow:*
  At step 12, take the tube from the centrifuge and transfer with the minipet 200 µl of supernatant into one millilitre (1ml) of "Dilution Buffer 1X" ready to use in the provided kidney buffer tube. Mix with the minipet 3 to 5 times and transfer 200 µl of dilution into the reagent vial. Follow instructions in step 13.
- *For **lever** samples tissue only, dilute 3 times more the supernatant to set the detection sensitivity at the appropriate lever MRL (300ppb) and proceed as follow:*
  At step 12, take a provided tube containing one millilitre (1ml) of "Dilution Buffer 1X" ready to use and eliminate 200 µl of buffer with the minipet. Transfer with the minipet 400 µl (2 x 200 µl) of supernatant in the remaining 800 µl of buffer. Mix with the minipet 3 to 5 times and transfer 200 µl of dilution into the reagent vial. Follow instructions in step 13.
- For **Seafood** samples do not forget to withdraw the shell from the seafood body.

Urine sample - Remarks for TM00629 & TM00630.

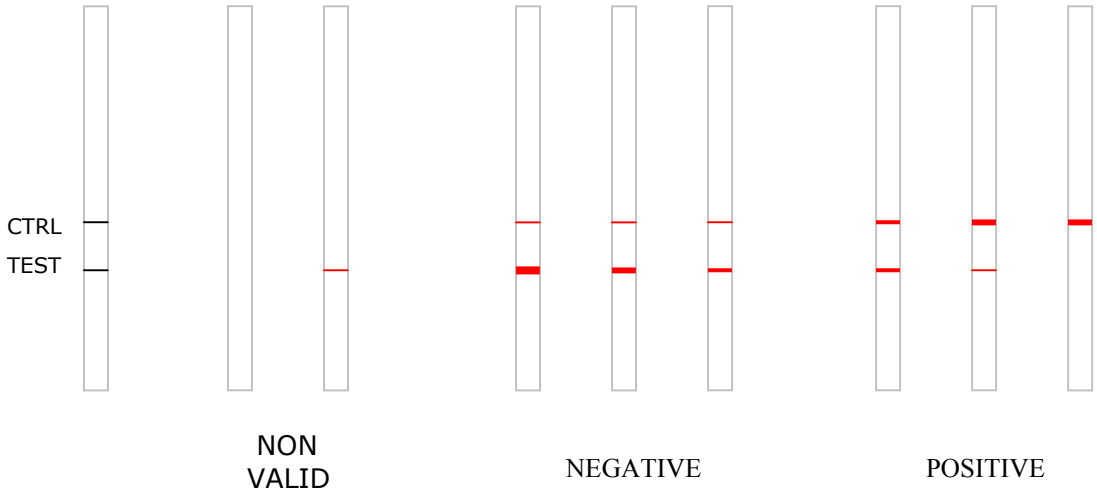
Liquid urine can be analysed only with kits TM00629 and TM00630. Urine must be diluted 4 times by mixing 200µl of urine sample with 600 µl of Urine Buffer supplied ready to use. For urine sample, pay attention of the conductivity and if conductivity of diluted urine is higher than 10 mS, dilute more to lower conductivity below 10mS. Take into account of this additional factor like follow: A 4 times dilution gives a limit as mentioned on top of the kit (20ppb) but a 2 times more dilution gives a limit of detection of 40 ppb (2 x 20ppb), a 4 times more dilution gives a limit of detection of 80 ppb (4 x 20ppb).

- *For **Urine** samples dilute 4 times and proceed as follow:*
  Take 200µl of urine and mix with 600µl of "Urine Buffer 1 X" ready to use. Start in step 9 and in step 12 transfer 200µl of this mixture into reagent vial and follow like in step 13.

Eyes Interpretation of the test.

Comparing the intensity between the bottom «TEST line» and the upper weak «CTRL line» does eyes interpretation of the result.

- If no red line occurs, the test is non valid.
- As being valid, the upper control (CTRL) line must turn to red.
- If the bottom « TEST » line is more visible than the upper « CTRL » line, the sample contains less tetracycline than the detection limit of the concerned kit (Sample is considered being NEGATIVE).
- If the bottom « TEST » line is as visible or less visible than the upper « CTRL » line, the sample contains as much - or more - tetracycline than the detection limit of the concerned kit (Sample is considered being POSITIVE).
- No bottom « TEST » line indicates that the sample contains far more tetracycline than the detection limit of the concerned kit (HIGHLY POSITIVE SAMPLE).
- When hesitating, consider POSITIVE and confirm the reading 30 minutes later.



Measurement with QuantiSensor.

To get a more objective interpretation of the result, the developed dipstick can be immediately measured with the optical "Quantisensor". We recommend to make the measurement within 30 minutes after the end of the procedure.
In 3 mouse clicks the result appears on your screen and the information is definitively stored in your personal computer. The generated analytical report including dipstick picture, integrates the result of the optical measurement together with additional information (sample identification, reagents lot number, operator name, date, …). At any time you can either recover or print any specific report or measurement. Moreover files of results can be electronically exported to any central data logger.

Please refer to the Quantisensor manual to proceed correctly.

References.

Okerman *et al.*, Evaluation and establishing the performance of different screening tests for tetracycline residues in animal tissues. Food Addit Contam. 2004 Feb;21(2):145-53.

Alfredsson G et al., Simple and rapid screening and confirmation of tetracyclines in honey and egg by a dipstick test and LC-MS/MS. Anal Chim Acta. 2005;529(1-2):47-51.