Enrofloxacin extraction kit

Catalog number: 20141
40 Tests
For Research Use Only. This kit has not been validated for diagnostic purposes.

Certo Labs Inc.
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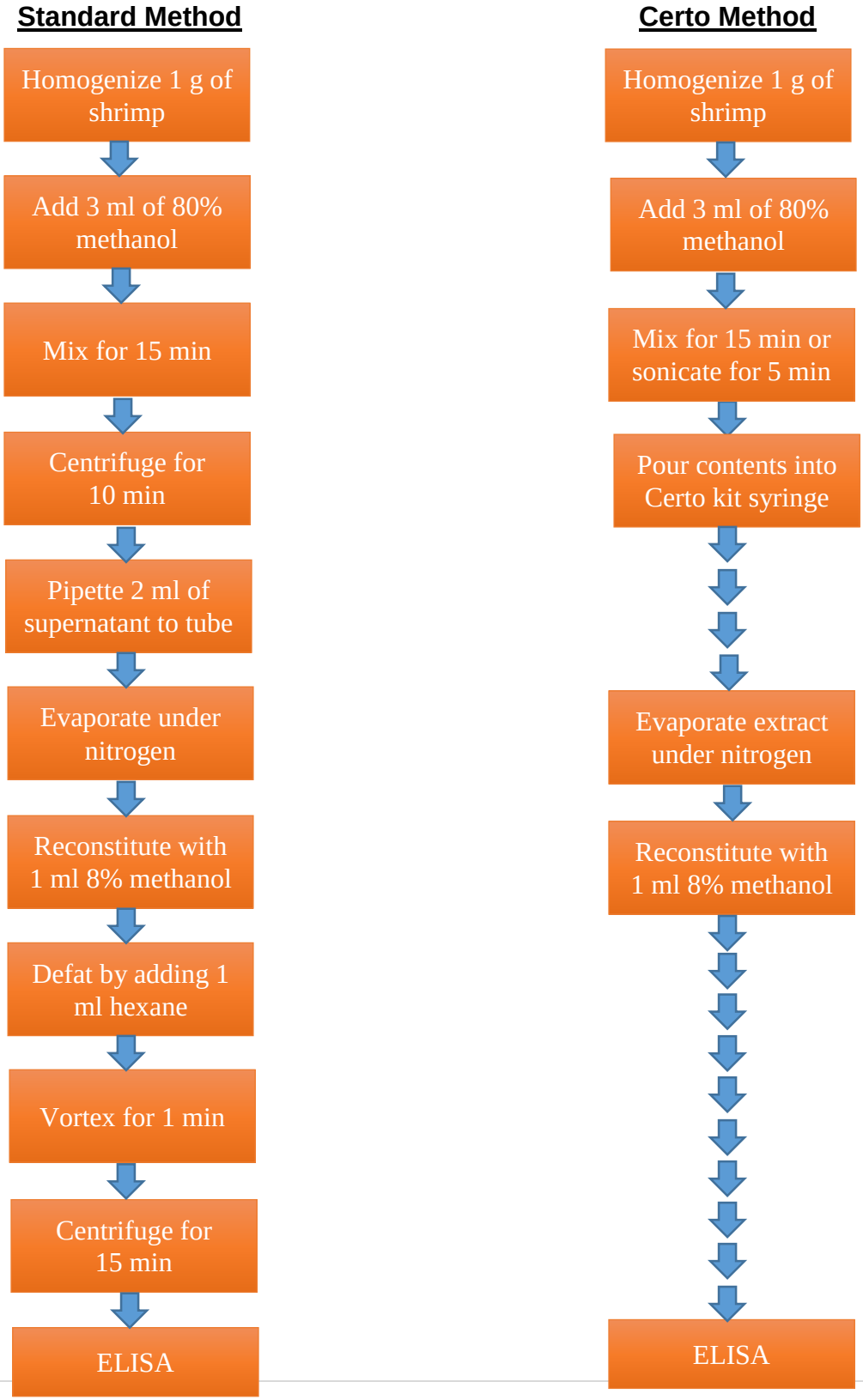
1. Intended purpose

The Certo Labs enrofloxacin extraction kit is intended for the extraction of enrofloxacin from food and biological samples.

2. Introduction

Enrofloxacin is a fluoroquinolone antibiotic used for the treatment of pets and domestic animals and in fish farming. Minimum acceptable limits have been set for enrofloxacin residues in food samples. The Certo enrofloxacin extraction kit enables the simultaneous separation of enrofloxacin from tissue, and the defatting of the sample during extraction in an accurate and quick manner. As shown in **Figure 1**, the Certo process bypasses the centrifugation, vortexing, pipetting and solvent addition steps associated with extracting and defatting the sample. The extracted enrofloxacin can then be detected and quantified by enzyme-linked immunosorbent assay (ELISA) followed by spectrophotometric analysis.

Figure 1: Standard and Certo method comparison



3. Principle:

General Certo principle: Homogenize → Extract → Reconstitute → ELISA

Enrofloxacin is typically extracted using a single solvent system containing 80% methanol in buffer. In the standard procedure, methanol containing the enrofloxacin is separated from tissue by centrifugation for 10 minutes followed by manual pipetting. The extract is then dried and reconstituted into 1mL each of 8% methanol in sample dilution buffer. The extract is subsequently defatted by adding 1 mL hexane and centrifuging. A 50 μL aliquot is taken from the bottom layer containing the defatted enrofloxacin extract and used for analysis by ELISA and spectrophotometry.

The Certo Enrofloxacin Extraction kit shortcuts the extraction process by eliminating the need for centrifugation, manual pipetting, and post-extraction defatting with hexane or another lipophilic solvent. After adding 80% methanol to the sample, it can be mixed manually for 15 minutes or with sonicated for 5 minutes, and poured through the Certo syringe containing a filter. The eluted solution containing the enrofloxacin extract is then reconstituted in 8% methanol and a 50 μL aliquot is used for analysis by ELISA and spectrophotometry.

Data comparing the standard chloramphenicol extraction method to the Certo kit extraction method is presented in **Figure 2** of section 14.

4. Warnings and precautions

- All reagents within the Certo Enrofloxacin Extraction Kit are intended for research purposes.
- The kit contains methanol, which is highly flammable and toxic if inhaled, ingested or contacted with the skin. These solvents must be handled with gloves, eye protection, appropriate protective clothing and under a fumehood. In case of an accident, contact a physician immediately.
- Wear gloves while handling the kit and wash hands thoroughly afterwards.
- Do NOT pipette by mouth.
- Do not eat, drink, smoke or apply makeup in areas where the kit is handled.

5. Materials provided

Article	Designation	Amount
Extraction solvent	**Methanol**	**96 ml**
Reconstitution solvent	**Methanol**	**42 ml**
Syringe containing filter	**Filter**	**40**
Syringe plunger	**Plunger**	**40**

6. Additional special equipment

- Homogenizer to homogenize solid samples.
- Pyrex glass tubes to collect the enrofloxacin extract.
- Spectrophotometer, capable of detecting 96 well plates at a wavelength of 450 nm.
- Europroxima chloramphenicol ELISA kit (Catalogue# 5101ERFX) or a similar kit**
    - **Note: The Certo Labs Enrofloxacin Extraction Kit has been tested with the Europroxima Enroflocaxin ELISA kit. If an alternate kit is utilized it is recommended that this be tested accordingly.

7. Reagent preparation

None.

8. Specimen

Any biological food sample – shrimp, meat, liver, crab, fish.

9. Procedure

Enrofloxacin concentrations (ng/g) can be derived from biological specimens. The Certo kit enables the extraction of enrofloxacin for quantitation by ELISA.

10. Sample preparation

1. Homogenize approximately 10 g of tissue.
2. Weight 1g of homogenized sample and transfer it to a test-tube.
3. Add 3 ml of 80% methanol (see reagent preparation above).
4. Mix head over head for 15 min or with ultrasound sonication for 5 minutes.
5. Pour the solution into the syringe containing the filter. *NOTE: The syringe should be placed on top of a collecting tube (Falcon tube, pyrex tube, etc.) that can hold at least 4 ml of solvent.*

6. Push the plunger to elute solvent into the collecting tube. The eluted solvent contains the defatted enrofloxacin extract in methanol.
7. Evaporate the methanol under a stream of nitrogen at 50°C.
8. Dissolve the remaining residue in 1 mL of 8% methanol (see reagent preparation above)
9. Pipette 50 µL into individual wells of the ELISA kit. *NOTE: in the case of an emulsified upper layer, heat the test-tube in a water bath at 80°C for 5 min and re-centrifuge.*
10. Enrofloxacin extract may now be analyzed by ELISA as described in the Europroxima chloramphenicol kit (Catalogue# 5101ERFX).

13. Disposal

The syringe, filter and plunger must be disposed in appropriate chemical waste containers after use. They cannot be used more than once.

14. Data comparing standard method to the Certo kit method.

Figure 2: Enrofloxacin levels in shrimp samples spiked with 5 ng / g of enrofloxacin. Enrofloxacin was extracted from shrimp samples with the standard or Certo kit methods shown in Figure 1, and quantified with ELISA.