3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Enrofloxacin Extraction Kit

Catalog Number **MAK226**
Store at Room Temperature

TECHNICAL BULLETIN

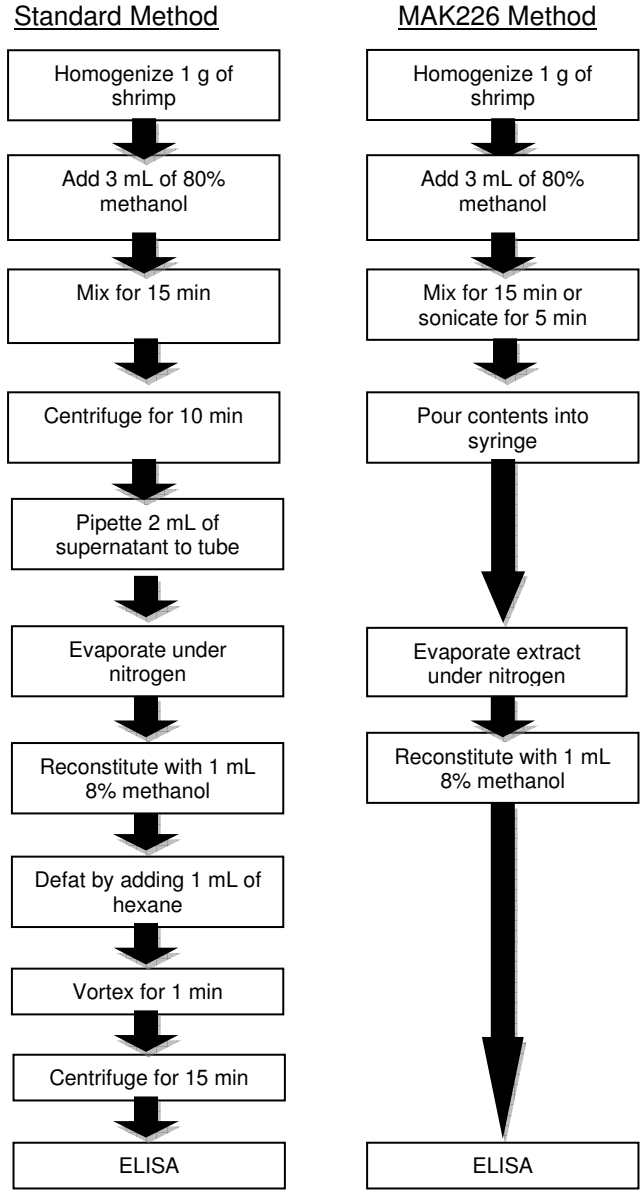
Product Description
The Enrofloxacin Extraction Kit is intended for the extraction of enrofloxacin from food and biological samples. Enrofloxacin is a fluoroquinolone antibiotic used for the treatment of pets and domestic animals and in fish farming. Minimum acceptable limits have been set for enrofloxacin residues in food samples. This kit can be used for any biological food sample - shrimp, meat, liver, crab, fish, etc.

The 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 streamlined 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.

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 1 mL 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 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 sonication for 5 minutes, and poured through the 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.

Figure 1: Standard Enrofloxacin Extraction and MAK226 Enrofloxacin Extraction method comparison



Components
The kit is sufficient for 40 Assays.

Extraction solvent Catalog Number MAK226A	122 mL
Reconstitution solvent Catalog Number MAK226B	42 mL
Plunger Syringe w/ Filter Catalog Number MAK226C	40 ea

Reagents and Equipment Required but Not Provided.

- Homogenizer to homogenize solid samples.
- Pyrex® glass tubes to collect the enrofloxacin extract.
- Spectrophotometric multiwell plate reader, capable of detection at 450 nm.
- Europroxima enrofloxacin ELISA kit (Catalog No. 5101ERFX) or a similar kit.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the kit at room temperature.

Procedure
Read entire protocol before performing the assay.

This kit enables the extraction of enrofloxacin for quantitation by ELISA. Enrofloxacin concentrations (ng/g) biological specimens for can be determined with use of the appropriate ELISA kit.

1. Homogenize approximately 10 g of tissue.
2. Weigh 1 g of homogenized sample and transfer it to a test-tube.
3. Add 3 mL of Extraction solvent. Mix by inversion for 15 minutes or with ultrasound sonication for 5 minutes.
4. Pour the solution into the syringe containing the filter.
   Note: The syringe should be placed on top of a collecting tube (e.g. Pyrex glass tube) that can hold at least 4 mL of solvent.
5. Push the plunger to elute solvent into the collecting tube. The eluted solvent contains the defatted enrofloxacin extract in methanol.
6. Evaporate the methanol under a stream of nitrogen at 50 °C.
7. Dissolve the remaining residue in 1 mL of Reconstitution solvent.
8. 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 minutes and centrifuge for 10 minutes at 2,000 × *g*.
9. Enrofloxacin extract may now be analyzed by ELISA as described in the Europroxima Enrofloxacin kit (Catalog No. 5101ERFX).
10. The syringe, filter, and plunger must be disposed in appropriate chemical waste containers after use. They cannot be used more than once.

PYREX is a registered trademark of Corning, Inc.

SS,SJ,TMS 06/20-1