

Helping Create Nutritious, Safe And Sustainable Seafood  
For Consumers Of Tomorrow

## Polycyclic Aromatic Hydrocarbon Test Solution

### Novel Qualitative Enzyme Inhibition Screening Assay



#### Key Benefits

- Easy to use- no professional training required
- No laboratory required- the kits can be easily implemented in an industrial setting or in the field
- Cost effective- no need for expensive reagents or equipment
- Complies with regulatory limits
- Validated for seabass & salmon

#### Assay Information

- Assay time-60 mins/96 well-plate
- Sample preparation time-20 samples/6hours
- Precision & Accuracy- less than 5% false negatives
- No interference above maximum residue limits for: PFOS, PFOA, BFRS, Dioxins, PCBS, Pesticides

#### Cross Reactivity

- Benzo(a)pyrene 100% (reference standard)
- Benzo(k)fluoranthene -125%
- OH-pyrene - 25%
- Phenanthrene - 7%
- Fluorene - 5%
- Acenaphthylene - 0.5%

#### Limits of Detection (LODs)

- Benzo(a)pyrene: 2µg/kg
- PAH4 (sum of benzo(a)anthracene, chrysene, benzo(b)fluoroanthere and benzo(a)pyrene: 10µg/kg

## Step Process of Sample Preparation



Step One

Weigh 7.5/12.5 g of homogenized fish sample



Step Two

Add 12 mL of acetonitrile and 3 mL of ddH<sub>2</sub>O



Step Three

Add QuEChER to sample tube & vortex for 2 minutes



Step Four

Centrifuge at 3500 rpm for 10 minutes at room temperature



Step Five

Transfer the supernatant to a glass tube containing NaCl and add 5 mL of water to the supernatant & vortex



Step Six

Incubate diluted supernatants in freezer for 30 minutes



Step Seven

Transfer all supernatant (organic phase) into clean glass tube and dilute 2 fold using ddH<sub>2</sub>O



Step Eight

Precondition a C18 500mg cartridge and pass all diluted supernatant through the cartridge



Step Nine

Elute using Acetonitrile and filter through a PSA/ Neutral aluminium oxide column



Step Ten

Evaporate Acetonitrile under gentle stream of Nitrogen at room temperature



Step Eleven

Reconstitute sample in acetone and phosphate buffer and vortex, centrifuge and dilute 25 fold. Sample is ready for application to test device

## Step Process of Assay Detection

Prepare Reaction Mixture 1 using Reagent A & B

1

Add a 80  $\mu$ L of sample extract to each well

2

Add 10  $\mu$ L of Reaction Mixture 1 per well and incubate for 5 minutes at 37°C and shake for 8 minutes

3

Measure the fluorescence of each well

6

Remove microtitre plate from incubator and add 10  $\mu$ L of Reaction Mixture 2 to every well. Incubate for 60 minutes at 37°C

5

Prepare Reaction Mixture 2 using phosphate buffer, Reagent C & Reagent D

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