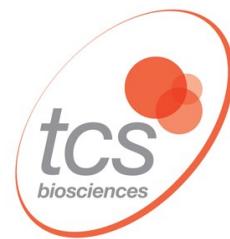


Isolate[®]



cryptosporidium

Step by step instructions



www.tcsbiosciences.co.uk

Isolate[®] for IMS of *Cryptosporidium* oocysts

- Semi Automated Immunomagnetic Separation
- Yields High Recoveries in Turbid Water Samples
- Processes Pellets up to 2ml in Volume

Isolate[®] offers unique, semi automated immunomagnetic separation (IMS) of *Cryptosporidium* oocysts in environmental water samples. The unique automation of the Isolate[®] test standardises the rocking motion, resulting in improved consistency and reliability of results.

Isolate[®] yields increased recoveries of oocysts and produces good results even when processing turbid water samples. In addition Isolate[®] is able to process pellets up to 2 ml in volume, reducing the need for split samples and multiple slides.

Cryptosporidium oocysts in the concentrate are captured and separated by magnetic action. Separated oocysts are stained with an approved FITC antibody stain, and visualised by fluorescence microscopy.

Isolate[®] can:

- Offer equivalent or better recoveries compared to your existing test
- Reduce overall test time due to multiple sample processing
- Reduce variation in recoveries due to automation
- Eliminate the risk of RSI
- Enable customers to greatly reduce their IMS costs

Isolate[®] is:

- Given excellent support by TCS Biosciences Ltd
- Despatched along with monthly EasyStain[™] and EasySeed[™] requirements



Isolate[®] materials provided:

- Crypto Magnetic Beads
- Reagent A - sample diluent
- Reagent B - blocking buffer
- 100 tests per kit

Equipment Required:

- Variable Speed Rotator
- Isolate[®] Rotator Disc and Hub
- Incu-Clips
- Magna-Clips
- Micro-Clips
- Dissociation Block
- Leighton Tubes



Isolate[®] oocyst capture procedure

Step 1



- Label the Leighton tube with sample number
- Add 1ml of Reagent A and Reagent B
- Add the sample that is to be tested
- Rinse the centrifuge tube with 1ml of purified water

Step 2



- Mix the Crypto Magnetic Beads
- Add 100µl of Crypto Magnetic Beads
- Ensure Leighton tube is properly closed
- Ensure rotator angle is 20° from horizontal

Step 3



- Place the Leighton tube in the blue Incu-Clip
- Slide the Incu-Clip into the rotator
- Insert up to six Incu-Clips and tubes
- Start the rotator at 20 RPM
- Leave for one hour at room temperature

Step 4



- Remove Incu-Clip(s) from rotator
- Remove Leighton tube from Incu-Clip
- Place tube in white Magna-Clip
- Slot up to six Magna-Clips onto rotator

Step 5



Start rotator

Rotate samples:

- < 0.5ml pellet for five minutes at room temperature,
- ≥ 0.5ml pellet for 10 minutes at room temperature

Step 6



Remove a single Magna-Clip from the rotator

Restart the rotator

With the tube in the Magna-Clip remove the cap from the tube

Step 7



Pour off the supernatant over the magnets

Without disturbing the beads, aspirate any remaining supernatant from the tube

Remove the tube from the Magna-Clip

Step 8



Add 0.8ml of dilute Reagent A

Rinse the bead pellet from the back of the tube

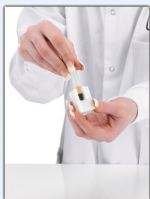
Repeat ensuring all beads are washed from the back of the tube

Transfer beads to a labelled microcentrifuge tube

Rinse the Leighton tube with a further 0.2ml of dilute

Reagent A and transfer into the microcentrifuge tube

Step 9



Cap microcentrifuge tube

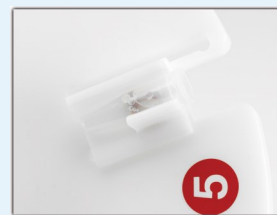
Slide the microcentrifuge tube into the Micro-Clip

Slot up to six Micro-Clips onto rotator

Start rotator

Rotate samples for: < 0.5ml pellet for two minutes at room temperature, ≥ 0.5ml pellet for four minutes

Step 10



Remove Micro-Clip from rotator

Restart rotator with remaining samples

Remove supernatant from the tube and cap, taking care not to disturb the beads

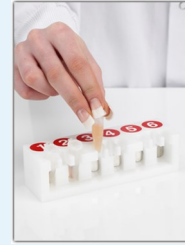
For turbid samples it may be advantageous to wash the beads a second time

Step 11



Remove microcentrifuge tube from the Micro-Clip
Add 50µl 0.1 N hydrochloric acid (HCl)
Vortex tube vigorously for ten seconds

Step 12



Stand tube for five minutes at room temperature
Repeat vortex
Insert tube(s) into the dissociation block

Dissociation of Oocysts

Step 13



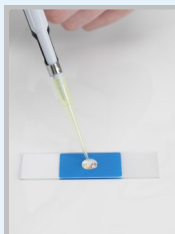
Lay the dissociation block flat on the bench so that the tube is horizontal with the magnet below
Leave for thirty seconds
Label a clean 9mm well slide
Add 5µl 1.0 N sodium hydroxide (NaOH) directly to sample well on slide
Carefully return the dissociation block to the upright position

Step 14



Add a further 5µl 1.0 N sodium hydroxide (NaOH) directly to the sample well slide
Transfer the supernatant from microcentrifuge tube to the well slide
Gently mix the sample using the transfer pipette

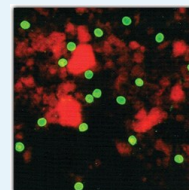
Step 15



Transfer the supernatant to the well slide
Gently mix sample using transfer pipette
Repeat HCl wash

Also available from TCS

- FITC conjugated antibodies *Cryptosporidium*/*Giardia*
- Enumerated quality control products *Cryptosporidium*/*Giardia*
- Inactive Bulk *Cryptosporidium* oocysts and *Giardia* cysts.



Isolate[®] benefits:

- Simple protocol
- Semi-automated procedure
- Multi-sample handling
- Fast throughput
- Standardised procedure
- Yields high recoveries in turbid water
- Processes water samples with up to 2ml packed pellets
- Improved recoveries

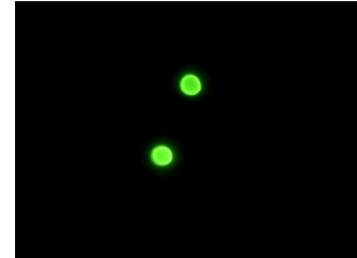


Image courtesy of Dr Guy Robinson *Cryptosporidium* Reference Unit UK



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