



## Neogen® Molecular Detection Assay (MDA) 2 – STEC Gene Screen (*stx* and *eae*) - AOAC 071902

### SCOPE

This method is applicable for testing of raw beef trim or raw ground beef for rapid screening of STEC genes (*stx1/stx2* and *eae*). This method is not applicable for testing *E. coli* O157:H7.

### PRINCIPLES

The Neogen® MDA 2 – STEC Gene Screen method is used for the rapid detection of *E. coli* genes *stx1* and/or *stx2* and *eae* in enriched foods. All samples identified as potentially positive for *stx* and *eae* genes using this method must be confirmed using a department approved confirmatory method.

The detection of STEC involves the following steps:

- **Sample enrichment**  
Meat samples (375g) are enriched in 1125 mL of pre-warmed (41.5 ±1°C) BPW ISO enrichment broth. The sample is homogenised (use of filter bags is recommended) for two minutes and incubated at 41.5 ± 1°C for 10-18 hours. A positive control culture must be run through all procedures daily or when testing is carried out. The sample and enrichment broth must be at the enrichment temperature for minimum of 10 hours.
- **Neogen® Molecular Detection System for screening STEC genes**  
STEC is screened in the sample following the manufacturer's recommended protocol. Neogen® Molecular Detection Assay 2 – STEC Gene Screen (*stx* and *eae*) method uses isothermal amplification of unique DNA target sequences in STEC virulence genes, *stx* and *eae* and bioluminescence to detect the amplified sequences. Positive samples will be considered as potential positive.
- **Confirmation**  
In all cases of a Neogen -positive, Neogen-inspect or Neogen-signal-error, the enrichment broth must be confirmed for the presence of top seven STEC at a department approved confirmatory laboratory using a department approved confirmatory method.

**CHECKLIST**

<b>Enrichment</b>	Is the Neogen BPW ISO enrichment broth warmed to $41.5 \pm 1^\circ\text{C}$ before use?	_____
	Is the correct amount of broth used for the weight of sample analysed i.e. 1125 mL?	_____
	Is a positive control culture run with each batch of samples analysed?	_____
	Are control cultures inoculated into the enrichment broth at a level of 10 to 100 cells?	_____
	Is enrichment carried out at $41.5 \pm 1^\circ\text{C}$ and is the enrichment broth and sample at $41.5 \pm 1^\circ\text{C}$ for a minimum of 10 hours?	_____
<b>Screening</b>	Are the manufacturer's instructions reproduced in the laboratory manual and followed without modification?	_____
	Are technicians familiar with and trained in the operation of the Neogen® Molecular Detection System?	_____
	Is the shelf-life of media, reagents and kits controlled?	
	Are the Neogen Molecular Detection Assay 2 kits stored at $2-8^\circ\text{C}$ ?	
	Are open kits used by 90 days?	
	Are open kits kept in resealed pouches with desiccant inside and stored at $2-8^\circ\text{C}$ ?	_____
<b>Confirmation</b>	Is STEC confirmed at a department approved laboratory using a department approved confirmatory method?	_____