



Solus *Salmonella* ELISA – NF SOL 37/01 – 06/13

SCOPE

This method is applicable to food and feed products and environmental samples. This method is not validated on food samples weighing more than 25 g.

PRINCIPLES

Detection of *Salmonella* is based on enzyme-linked immunosorbent assay (ELISA). *Salmonella* specific antibodies are coated on the wall of microplate wells that attach with *Salmonella* antigens when present in the enrichment. After washing an enzyme-labelled antibody is added to the well which then binds with the antigen-antibody complex. After the second wash, a substrate is added which in the presence of bound enzyme-antigen-antibody complex produces blue colour. The substrate reaction is stopped after 30 seconds by acid and the intensity of the colour is measured. The detection protocol can be broken down as follows:

- **Pre-enrichment**
Raw meat sample (25 g) is enriched in 225 mL pre-warmed (room temperature or $37 \pm 1^\circ\text{C}$ for large volumes) buffered peptone water (BPW). For carcass sponges, buffered peptone water is added to the moistened sponge to bring the total volume to 225 mL.

Homogenized matrices and incubated at $37 \pm 1^\circ\text{C}$ for 16 - 20 h. A positive control culture must be run through all procedures daily or when testing is carried out.
- **Selective enrichment**
Subculture of 0.1 mL pre-enrichment is inoculated in 10 mL of RVS and incubated $41.5 \pm 1^\circ\text{C}$ for 24 ± 3 h.
- **Enzyme immunoassay**
Solus ELISA is performed using a portion of boiled enrichment broth. The assay is carried out in the Solus instrument following the manufacturer's instructions.
- **Cultural confirmation**
All *Salmonella* positive samples should be confirmed using enrichment broth following AS 5013.10. Confirmation carried out at an 'off-site' laboratory must be from retained BPW enrichment.

CHECKLIST

Enrichments	Is correct amount of BPW used for the weight of sample analysed?	_____
	Is BPW warmed prior to use (room temperature or 37°C)?	_____
	Is a positive control run with each batch of samples analysed?	_____
	Are reference cultures inoculated into primary enrichment broth at a level of 10 to 100 cells?	_____
	Is pre-enrichment done at 37 ± 1°C for 16 - 20h & selective enrichment at 41.5 ± 1°C for 24 ± 3h?	_____
Enzyme immunoassay	Are the manufacturer's instructions available?	_____
	Are ELISA kits allowed one hour to warm up at room temperature before use?	_____
	Are positive & negative kit controls run with each batch of samples?	_____
	Are plates incubated at 37°C for 30 min after adding samples and again after adding conjugate?	_____
	Are ELISA plates washed using a microplate washer?	_____
	Are OD readings taken within 10 min of adding stop solution?	_____
	Are samples with OD ₄₅₀ ≥ 0.200 considered presumptive positive?	_____
	Are ELISA kits stored as per manufacturer's guidelines (2 -8°C)?	_____
Cultural confirmation	Is <i>Salmonella</i> confirmed from RVS broth by the laboratory?	_____
(if applicable)	If not, is BPW supplied to off-site laboratory for confirmation?	_____
	Are <i>Salmonella</i> confirmed at a department approved lab using AS 5013.10 (with appropriate selective agar plates)?	_____