

## ENDOTOXIN CHALLENGE VIALS

### CATALOG NUMBER

ECV100000V

### INTENDED USE

The Endotoxin Challenge Vial (ECV) is used in the validation of dry heat depyrogenation cycles. The ability of a particular oven cycle to destroy/inactivate endotoxin is measured by comparing the endotoxin level(s) in baked ECVs vs unbaked control ECVs. The United States Pharmacopeia (USP) recommends that in order for a depyrogenation process to be valid, the endotoxin content of a challenge vial must be reduced at least 1000 fold ( $>3$  log cycle reduction). The ECVs might be used with Gel Clot Amebocyte Lysate, Kinetic Turbidimetric Amebocyte Lysate, Kinetic Chromogenic Amebocyte Lysate or End-point Chromogenic Amebocyte Lysate endotoxin assays.

### REAGENT

Endotoxin Challenge Vial (ECV) containing  $\geq 100,000$  EU of purified *E. coli* O111:B4 endotoxin.

STORAGE: Store vials at 2 to 8°C with closures and stoppers intact.

### NOTE

ECVs may appear to be empty due to the extremely small amount of endotoxin per vial. This “empty” appearance is normal.

### WARNING

This product contains pyrogenic amounts of endotoxin.

Endotoxin Challenge Vials are for *In Vitro* use only, and not to be used in humans or animals.

### PRELIMINARY PREPARATION

**Note: Vials labels are heat resistant and do not need to be removed prior to depyrogenation, the seal caps and stoppers are also heat resistant, and not need to be removed prior to depyrogenation.**

Reconstitute the ECV vial by adding 4.0 ml Water for BET. Vortex for at least 15 minutes. The endotoxin in reconstituted vials will be stable for 10 days if store at 2-8°C.

Transfer 0.1 ml of reconstituted endotoxin solution to each endotoxin-free vial, each vial will contain about 2500 EU endotoxin.

Place the appropriate number of endotoxin vials in the oven at the predetermined hardest-to-heat location(s).

Bake endotoxin vials according to user-selected cycle parameters.

After the depyrogenation cycle is complete, remove the vials for endotoxin testing. Reconstitute each baked vial with 1.0 ml of Water for BET. Add 0.9 ml Water for BET into each unbaked endotoxin vial.

Vortex at high speed for 15 minutes. Prepare the appropriate dilutions from the unbaked samples using Water for BET (see **TEST PRECEDURE AND CALCULATION OF RESULTS**). Test immediately using a gel clot, kinetic turbidimetric, kinetic chromogenic or end point chromogenic endotoxin assay.

## TEST PRECEDURE AND CALCULATION OF RESULTS

### Using gel clot endotoxin assay

Determine the concentration of endotoxin in unbaked control vials by diluting the reconstituted unbaked control endotoxin vials 5000, 10,000, 20,000, 40,000 folds using Water for BET.

Do not dilute the baked endotoxin vials.

Assay diluted unbaked endotoxin dilutions and baked endotoxin solutions using Amebocyte Lysate with a 0.25 EU/ml lysate sensitivity. The endotoxin concentration in the reconstituted endotoxin vial can be calculated as follows:

Endotoxin concentration

= lysate sensitivity (EU/ml) x reconstitution volume (ml/vial) x maximum positive dilution (Dilution refers to the denominator of the dilution fraction, e.g. for a 1/10,000 dilution, the denominator = 10,000. For undiluted samples, dilution = 1)

A positive reaction indicates an endotoxin content equal to or greater than the calculated endotoxin value.

A negative reaction indicates an endotoxin content less than the calculated endotoxin value.

A positive reaction in the 1/10,000 dilution of the unbaked control vials indicates an initial endotoxin concentration equal to or greater than 2500 EU/vial.

Example:

Endotoxin concentration = 0.25 EU/ml x 1 ml/vial x 10,000 = 2500 EU/vial

A negative reaction in the undiluted baked vials indicates a final endotoxin concentration less than 0.25 EU/vial.

lg reduction

= lg mean endotoxin concentration of the unbaked control vials  
- lg mean endotoxin concentration of the baked vials

The mean endotoxin concentration of unbaked control endotoxin vials is equal to or greater than 2500 EU/ml. If that of the baked endotoxin vials is less than 0.25 EU/ml, the lg reduction is greater than 4. If that of the baked endotoxin vials is equal to or greater than 0.25 EU/ml, the lg reduction is equal to or less than 4.

**Note: If the minimum requirement for lg reduction is 3, dilute the baked endotoxin vials for 10 folds.**

**Using Kinetic Turbidimetric, Kinetic Chromogenic or End-point Chromogenic**

## endotoxin assay

Dilute the reconstituted unbaked control vials 1/10,000 using Water for BET. Do not dilute the baked vials.

The endotoxin concentration in the unbaked vials can be calculated as follows:

Endotoxin concentration (EU/vial)

= Endotoxin concentration of test sample (from standard curve) x reconstitution volume x dilution factor

(For undiluted samples, dilution factor = 1)

The resulting Mean value from a diluted control endotoxin vial sample that falls on the standard curve can be used to calculate a corresponding endotoxin value. Using the above formula, the endotoxin concentration of the reconstituted vials can be calculated.

Example:

Endotoxin concentration

= 0.37 EU/ml (from standard curve) x 1 ml/vial x 10,000

= 3700 EU/vial

The resulting Mean value from an undiluted baked endotoxin vials that falls on the standard curve can be used to calculate the endotoxin concentration for that sample. Using the formula above, the endotoxin concentration of the baked endotoxin vial can be calculated.

Example:

Endotoxin concentration

= 0.21 EU/ml (from standard curve) x 1 ml/vial x 1

= 0.21 EU/vial

The log reduction in the above example would be calculated as follows:

Log reduction = log endotoxin concentration of the unbaked control vials - log endotoxin concentration of the baked vials

Log reduction = log 3700 EU/vial - log 0.21 EU/vial

= 3.568 - (-0.678)

= 4.25

If the resulting Mean value from an undiluted baked endotoxin vials is less than the Mean value of the lowest standard, use the value of the lowest standard as the endotoxin concentration of the sample. The calculated log reduction will be the minimum log reduction.

**NOTE: To limit the log reduction to 3 logs, assay a 1/10 dilution of the reconstituted baked vials.**