BIOENDO

Xiamen Bioendo Technology Co., Ltd.

ENDOTOXIN CHALLENGE VIALS

CATALOG NUMBER
ECV2500A, ECV2500V

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 ≥2500 EU of purified *E. coli* O111:B4 endotoxin.
STORAGE: Store vials at 2 to 8℃ 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: ECV labels are heat resistant and do not need to be removed prior to depyrogenation. For ECV sealed in vials, the seal caps and stoppers are also heat resistant, and not need to be removed prior to depyrogenation.For ECV sealed in ampoules, do not need to open the vial.
Place the appropriate number of ECVs in the oven at the predetermined hardest-to-heat location(s).
Bake ECV vials according to user-selected cycle parameters.
After the depyrogenation cycle is complete, remove the vials for endotoxin testing.
For ECV2500A, which are sealed in ampoules, open the vials. Reconstitute each baked vial and an appropriate number of unbaked control ECVs with 1.0 ml of Water

for BET.
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 PROCEDURE AND CALCULATION OF RESULTS

Using gel clot endotoxin assay

Determine the concentration of endotoxin in unbaked control vials by diluting the reconstituted unbaked control ECV vials 5000, 10,000, 20,000, 40,000 folds using Water for BET.
Do not dilute the baked ECV vials.
Assay diluted unbaked ECV dilutions and backed ECV solutions using Amebocyte Lysate with a 0.25 EU/ml lysate sensitivity. The endotoxin concentration in the reconstituted Endotoxin Challenge Vials can be calculated as follows:
Endotoxin concentration
= lysate sensitivity x reconstitution volume (EU/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 ECVs is equal to or greater than 2500 EU/ml. If that of the baked ECVs is less than 0.25 EU/ml, the lg reduction is greater than 4. If that of the baked ECVs 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 backed ECVs 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 reconstituted ECVs 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 ECV 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 ECV can be calculated.

Example:
Endotoxin concentration
= 0.37 EU/ml (from standard curve) x1 ml/vial x 10,000
= 3700 EU/vial

The resulting Mean value from an undiluted baked ECV sample 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 ECV 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 ECV 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.

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