BOVIGAM™ Pokeweed Mitogen

Stimulation antigen for the laboratory diagnosis of cell viability using bovine interferon-gamma tests

Catalog Number 5108777

Pub. No. MAN0019565 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Introduction

The Applied Biosystems™ BOVIGAM™ Pokeweed Mitogen (Cat. No. 5108777; PWM) is a stimulation antigen for the BOVIGAM™ TB Kit and BOVIGAM™ 2G TB Kit, consisting of a lectin of the American pokeweed (*Phytolacca americana*).
BOVIGAM™ Pokeweed Mitogen is a cell viability control and acts as a non-specific agent for the stimulation of certain T cell populations. The reconstituted solution contains no preservatives.

General instructions

PWM is used when performing the BOVIGAM™ TB Kit or BOVIGAM™ 2G TB Kit to stimulate whole blood samples. It is essential that all blood samples collected for the BOVIGAM™ TB Kit or BOVIGAM™ 2G TB Kit be taken from a cleaned site and transferred into sample tubes containing lithium heparin as an anticoagulant. Invert the sample tubes several times to ensure that the blood and heparin are completely mixed. Blood samples should be transported to the test laboratory at room temperature (22±3°C, avoid extremes) and used in BOVIGAM™ assays within 30 hours of collection.
IMPORTANT! Blood must not be stored in a refrigerator.

Test principle

PWM is a suitable agent for non-specific stimulation of cell-mediated interferon-gamma (IFN-γ) production. Therefore, PWM can be used as a positive control for stimulation assays of this type.
PWM is mixed with lymphocytes in whole blood and incubated overnight. Blood plasma is then collected from above the cultures and assayed for IFN-γ production using the BOVIGAM™ TB Kit or BOVIGAM™ 2G TB Kit.

General precautions

Laboratory safety

National Safety Regulations must be strictly followed.

Test procedure

For stimulation in a 96-well format

The PWM is lyophilized and must be stored at 5±3°C. Prepare a stock solution (1 mg/mL) by reconstituting the lyophilized PWM with 3.2 mL of RPMI 1640 medium. The stock solution should be frozen in suitable aliquots at –25°C to –20°C.

For the stimulation, thaw suitable aliquots of the stock solution. In order to achieve a final concentration of 5 µg/mL for each assay, dilute 55 µL of the stock with 945 µL of RPMI 1640 medium (working concentration).

Mix blood samples evenly immediately before use. For the test, add 25 µL of PWM (from the working concentration) to the appropriate well, then add 250 µL of whole blood. Carefully mix by pipetting up and down.

For stimulation in a 24-well format

The PWM is lyophilized and must be stored at 5±3°C. Prepare a stock solution (1 mg/mL) by reconstituting the lyophilized PWM with 3.2 mL of RPMI 1640 medium. The stock solution should be frozen in suitable aliquots at –25°C to –20°C.

For the stimulation, thaw suitable aliquots of the stock solution. In order to achieve a final concentration of 5 µg/mL for each assay, dilute 80 µL of the stock with 920 µL of RPMI 1640 medium (working concentration).

Mix blood samples evenly immediately before use. For the test, add 100 µL of PWM (from the working concentration) to the appropriate well, then add 1.5 mL of whole blood. Carefully mix by pipetting up and down.

Collecting plasma samples

- 96-well plate: Centrifuge at 500 x *g* at room temperature for 10 minutes. Transfer 110 µL of the supernatant to a new 96-well plate. Seal the plate, then store at –80°C until the EIA is performed.
- 24-well plate: Transfer the required amount of plasma to a new 96-well plate, seal the plate, and then store at -80°C until the EIA is performed.

Perform the BOVIGAM™ TB Kit or BOVIGAM™ 2G TB Kit assay according to the respective *Instructions for Use* provided with the kit.

Interpretation

Stimulation with PWM (stimulation control)

1. For each sample, calculate the mean absorbance values for the nil antigen and PWM.
2. For each animal, compare the mean absorbance values of the nil antigen and PWM.

$$\text{Positive viability} = \text{OD PWM} - \text{Nil Antigen} \geq 0.5$$

$$\text{Negative viability} = \text{OD PWM} - \text{Nil Antigen} < 0.5$$

PWM-stimulated blood samples with an OD of <0.5 OD units (PWM-nil) are classified as samples that contain blood cells with insufficient viability. It is recommended to repeat the blood collection.

Whole blood samples that, after PWM stimulation, have a mean OD value (after subtracting the nil antigen value) greater than 0.5 contain vital lymphocytes.

Lower sample values can be an indication that the vitality of the lymphocytes was reduced due to transport or improper handling. PWM samples from stressed, malnourished, or immunosuppressed animals can also display OD values below 0.5 OD units. Samples that do not achieve this threshold should not be analyzed.

CAUTION

Immunosuppression caused by recent dexamethasone treatment or parturition may depress IFN-γ responses to mycobacterial antigens. Animals that have received an injection of dexamethasone within one week, or that have calved within 4 weeks, should be retested to reduce the possibility of a false-negative result.

Responsibility for the test interpretation and consequent animal husbandry decisions rests solely on the user, the consulting veterinarian, and the responsible health advisor.

Thermo Fisher Scientific accepts no responsibility for any loss or damage, howsoever caused, arising from the interpretation of the test results.

Retesting

Animals with a PWM-nil OD value <0.5 OD units should be retested.

Storage

Store protected from light at 5±3°C (lyophilized). Store reconstituted and aliquoted PWM at –20°C.

Mode of issue

BOVIGAM™ Pokeweed Mitogen is available in 3.2 mg tubes.

References

Schiller, I, Waters, WR, Vordermeier, HM, Nonnecke, B, Welsh, M, Keck, N, Whelan, A, Sigafoose, T, Stamm, C, Palmer, M, Thacker, T, Hardegger, R, Marg-Haufe, B, Raeber, A, and Oesch, B. *Clin. Vaccine Immunol.* 16(8), 1196-1202, 2009.

The procedure described here does not comply with the specifications in these instructions for use. Thermo Fisher Scientific is not responsible for results obtained using this procedure.

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Revision history of Pub. No. MAN0019565 (English)

Rev.	Date	Description
B.0	4 December 2023	The FLI registration information was removed from the document.
A.0	26 October 2020	New document translated from the German document (MAN0019564 Rev. A.0).