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	Title:	**TECHNICAL DATASHEET**		

Phytohemagglutinin M (PHA-M)

Filtration, Treatment	Sterile filtered
Product Code	CDS-001-10ML
Shelf Life	24 months from DOM. After thawing, PHA-M is stable for at least 1 month at +2°C to +8°C.
Storage Temperature	-20 to -5°C. After thawing, the PHA-M is stable for at least 1 month at +2°C to +8°C.
Shipping Temperature	Dry ice
Working concentration	2 ml of solution per 100 ml culture medium

QC Specifications

Physical and Chemical Analysis	**Method**	**Specifications**	**Units**
Appearance	Visual	Clear colorless frozen liquid	n/a
pH at RT	Electronic pH Meter	5.5 - 7.0	n/a
Endotoxin	LAL Kinetic	≤ 10.0	EU/ml
Sterility			
Aerobic Bacteria	Internally Validated	Not detected	n/a
Anaerobic Bacteria	Internally Validated	Not detected	n/a
Fungi (Yeast & Mold)	Internally Validated	Not detected	n/a
Cell culture			
Lymphocyte cytogenetics performance assay	Internally validated	Pass	n/a

General Information

Phytohaemagglutinin (PHA) is a lectin found in legumes and extracted from red kidney beans (*Phaseolus vulgaris*). Lectins are carbohydrate-binding proteins that bind specifically to the sugar groups of other molecules, which leads to agglutination of particular cells or precipitation of glycoconjugates and polysaccharides. PHA-M is the mucoprotein form and is used for the stimulation of mitosis and cell proliferation in lymphocyte culture. Peripheral blood lymphocytes are one of the most commonly used cells for karyotyping in modern human cytogenetics to detect chromosomal abnormalities. However, they are not dividing in a healthy adult, and to be used for karyotyping they should be treated with a mitogen. Therefore, PHA-M is used as a mitogen to stimulate mitosis by DNA replication in lymphocyte cells in a proper cell culture medium.

Serana PHA-M is a sterile and frozen solution whose cytogeneitcs performance for a proper lymphocyte karyotyping is validated using other relevant Serana cell culture products for each batch.

Applications

- Stimulates mitotic division of lymphocytes in cytogenetic and immunological applications
- Powerful erythroagglutinating properties

Instructions for Use

Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

In the presence of a mitogen (e.g. PHA), lymphocytes are stimulated to undergo mitosis. After 48 – 72 hours, a mitotic inhibitor (e.g., colcemid; Cat ID: CDS-002) is added to the culture to arrest mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

1. Add 2 ml of PHA-M per 100 ml of karyotyping medium (Cat ID: LMG-002).
2. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml karyotyping medium supplemented with PHA-M (or 10^6 viable cells per ml).
3. Incubate the culture at +37°C, 5 % CO2 in an incubator for 72 hours.
4. Add 0.1 – 0.2 ml of Colcemid Solution (Cat ID: CDS-002) to each culture tube (at a final concentration of 0.1 µg/ml). Incubate the culture for 30 minutes.
5. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.

6. Remove the supernatant and resuspend the cells in 5 – 10ml of hypotonic 0.075 M KCl (Cat ID: CDS-003), pre-warmed to +37°C. Incubate at +37°C for 15 minutes.
7. Spin at 500 g for 5 minutes.
8. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5 – 10 ml of fresh, ice-cold fixative (3:1-Methanol: Glacial acetic acid). Leave at +4 °C for 10 minutes.
9. Repeat steps 7 and 8.
10. Spin at 500 g for 5 minutes
11. Resuspend the cell pellet in a small volume (0.5 – 1 ml) of fresh fixative.
12. Drop the cells on a chilled glass slide and allow to dry.
13. At this stage, the preparation can be stained with Giemsa (Cat ID: CDS-004). you can use one of the common staining protocols or the protocol established in your laboratory.
14. Observe the mitotic arrested cells under phase contrast microscope.

Trouble Shooting

- PHA-M may appear cloudy at +2°C to +8°C. The turbidity has no effect on the activity of PHA-M.
- We highly recommend to use Serana`s Media, FBS, and reagents which were used in the product validation.

Precautions and Disclaimer

- For in vitro diagnostic use. The CDS-001 solution is not intended for therapeutic use.
- Each laboratory is obliged to perform representative tests according to the valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the solution can be used in routine diagnostics.
- Use of PHA-M does not guarantee the successful outcome of any diagnostic testing.

Do not use the product beyond the expiration date indicated on the product label