**Catalog No: CYT-PHA005**

# Phytohaemagglutinin (PHA)- Liquid form

## Principle

Peripheral blood is the most commonly utilized tissue for determination of constitutional karyotype. Peripheral blood lymphocytes are not dividing in normal, healthy adult; they must be stimulated to divide by exposure to mitogen.

The most commonly used mitogen for Cytogenetic studies is PHA. It is a mucoprotein isolated from seeds of Phaseolus vulgaris. It has the property of selectively agglutinating and sedimenting erythrocytes and of inducing division in leukocytes. When the T-lymphocytes are exposed to this plant antigen (PHA), initiates immunological response through behaviors resembling an antigen. The mature T cells dedifferentiate to a T-lymphoblastic cell, which has the capacity to synthesize DNA to under go mitosis. During the first 24 hrs exposure to PHA, the T cells undergo this transformation and as the culture duration increases undergo several rounds of cell division.

**Storage Conditions:** Store frozen at -20°C

After thawing PHA is stable for at least one month at +2/+8°C.

**Shelf Life** : 24 months

**Pack Size:** 5ml

## Recommended Use:

† Store the product at the mentioned temperature condition.

† Do not use the product after expiry.

† Use proper PPE at the time of handing product.

† The storage and work environmental should be free from humidity.

† The product is intended to used in laboratory only. Do not use in therapy, human or veterinary application.

## Final Concentration

† *2.5/3ml per 100ml of culture medium*
(Add 2.5/3ml of PHA in 100ml of peripheral blood culture medium for Karyotype)

**OR**

† *0.1/0.2ml per 5ml of culture medium*
(Add 0.1/0.2ml of PHA in 5ml of peripheral blood culture medium for Karyotype)

## Methodology-

### Working Medium Preparation

1) Add 200mg of sodium bicarbonate and 2.5/3ml of PHA in 100ml of peripheral blood plain culture medium (Preferably RPMI 1640).
2) Adjust the PH at 7.2 with 1N HCL/NAOH.

## Cell culture

3) Label the 15ml centrifuge tube with patient name and lab ID
   ***(Do not use culture flask, as it is close system culture)***
4) Add 5ml of working medium and 1ml of FBS
5) Add 0.3/0.5ml of heparinised whole blood sample
6) Incubate the cell culture at $37^0$C for 72 hrs (No need of CO2 incubator)

## Chromosome preparation

7) After completion of 72hrs add 0.2ml colcemid solution
8) Incubate the culture for another 30 minutes
9) Centrifuge the tube at 1000rpm for 10 minutes
10) Remove the supernatant and add 7-8ml hypotonic solution (0.075M KCL)
11) Incubate the tube at $37^0$C for 15mints
12) Add 5-6 drops of chilled fixative (3:1-Methanol: Glacial acetic acid), mix slowly
13) Centrifuge at 1000rpm for 10mints
14) Remove supernatant and add 7-8ml chilled fixative, mix it properly.
15) Repeat steps 13 and 14 for 3 times
16) In the final wash, will get white lymphocyte clear pellet

## Slide preparation

17) Re-suspend the cell pellet in a small volume of chilled fixative approx. 0.4 to 0.8ml
18) Drop the cells on chilled glass slide and allow to dry
19) Metaphases can be observe under phase contrast microscope

## Trouble Shooting

† PHA may appear cloudy at +2/+8$^o$C, but this turbidity has no effects on the activity of the products.

† Do not add more then 4ml of PHA per 100ml of medium

† Always run parallel culture before use of every lot

## Ref

1. D.E. Rooney, Human Cytogenetics constitutional analysis, Third edition, PP. 33-44.

2. Marilyn S. Arsham, Margaret J. Barch, The AGT cytogenetics Laboratory manual, Fourth edition, PP 91-100.

3. Verma RS, Babu A., Human Chromosome- Manual of Basic Techniques, 1988, PP.9-10.

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