FUJIFILM

IrvineScientific

Phytohemagglutinin

Catalog No. 96691 **5 mL**

Glossary of Symbols*:

REF	Catalog Number
LOT	Lot Number
STERILE A	Sterilized using aseptic processing techniques (filtration)
	Expiration: Year - Month - Day
	Caution, consult accompanying documents
	Consult instructions for use
8°C 2°C	Storage Temperature 2-8°C
	Do not use if package is damaged

*Symbol Reference - **EN ISO 15223-1,** Medical devices – Symbols to be used with medical device labels, labeling.

INDICATION FOR USE

Phytohemagglutinin may be used to stimulate mitotic division of lymphocytes maintained in a cell culture system and facilitate cytogenetic studies of the chromosomes.

DEVICE DESCRIPTION

Phytohemagglutinin is an analyte specific reagent. The analytical and performance characteristics are not established.

SUMMARY AND EXPLANATION OF THE TEST[3,5,6]

Phytohemagglutinin (PHA) is the generic name given to aqueous extracts of the seeds of certain plants, notably *Phaseolus* ssp. Originally used for separating leukocytes from whole blood because of its powerful erythroagglutinating property[2], it was later found to cause progressive mitotic division of lymphocytes in tissue culture[4].

Subsequent experience has shown that PHA is the most universally effective and convenient stimulant of lymphocytes from many animal species. The widespread popularity of peripheral blood culture as a means of chromosome analysis has been largely dependent on this property.

The procedure involves establishing a suitable lymphocyte culture system to which PHA is added. This stimulates mitosis which is arrested in metaphase by the addition of Colcemid® (deacetyl methylcolchicine). At this stage the chromosomes are most clearly visible. The sample is treated with hypotonic solution which results in swelling of cells and erythrocyte lysis facilitating chromosome identification in suitably fixed and stained slide preparations.

STORAGE INSTRUCTIONS

The lyophilized material will retain its reactivity at least until the date shown on the label when stored at 2°C to 8°C.

Reconstituted material should be stored at 2°C to 8°C and used within one month. Material showing bacterial contamination should be discarded; slight turbidity sometimes develops in the absence of bacterial growth and has no deleterious effect on the properties of the PHA.

QUANTITY SUPPLIED

Phytohemagglutinin is a lyophilized preparation of an aqueous extract from selected seed beans of *Phaseolus* spp. Each bottle contains approximately 45 mg of lyophilized extract.

Phytohemagglutinin is manufactured for FUJIFILM Irvine Scientific.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. Microculture medium sufficient for 25 tests:
   100 mL RPMI Medium 1640
   25 mL Fetal Bovine Serum
   1.25 mL L-Glutamine (200 mM)
   0.35 mL Gentamicin Sulfate Solution (10 mg/mL) or use CHANG Medium MF in place of microculture medium
2. Phenol-free heparin
3. Colcemid 25 µg/mL solution
4. Potassium Chloride solution, 75 mM
5. Acetic alcohol, 1 part glacial acetic acid: 3 parts methanol (Analytical Reagent Grade)
6. Giemsa or 2% acetic acid-orcein
7. Mountant
8. Glass slides and coverslips
9. Plastic centrifuge tubes
10. CO_2 Incubator
11. Bench centrifuge
12. Vortex mixer
13. Light microscope

REFERENCES

1. Arakaki, D. T. and Sparkes, R. S. (1963), ***Cytogenetics***, 2**,** 57-60.
2. Li, J. G. et Osgood, E. E. (1949). Blood, 4**,** 670.
3. Maluish, A. E. and Strong, D. M. (1986). ***In*** Manual of Clinical Laboratory Immunology, pp. 274-281, 3rd Edition, Eds. N. R. Rose, H. Friedman and J. L. Fahey. ***American Society for Microbiology,*** Washington, D. C.
4. Nowell, P. C. (1960). ***Cancer Research*** 20, 462-466.
5. Waithe, W. I. and Hirschorn, K. (1978). ***In*** Handbook of Experimental Immunology, 3rd Edition, D. M. Weir, Ed. Blackwell Scientific Publications, Oxford, Chapter 26 - Lymphocyte responses to activators.
6. Watt, J. L. and Stephen G. S. (1986). ***In*** Human Cytogenetics: a practical approach, pp. 39-55. Eds.D. E. Rooney and B. H. Czepulkowski, IRL Press Ltd., Oxford.
7. A proposed Standard System of Nomenclature of Human Mitotic Chromosomes. (1960). ***Lancet, i,***1063.

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PRECAUTIONS AND WARNINGS
Do not use any bottle in which the sterile packaging has been compromised.

RECONSTITUTION AND PREPARATION OF REAGENT
Each bottle of lyophilized PHA should be reconstituted by the addition of 5 mL of sterile distilled water using a sterile disposable hypodermic syringe. The cap of the bottle should be sterilized by wiping with 70% ethanol, the needle should pierce the center of the rubber plug and be held in a vertical position during reconstitution.

SPECIMEN COLLECTION AND PREPARATION[6]
The success of whole blood cultures for cytogenetic analysis is influenced by the level of lymphocytes with normal function at the time of sampling. As this level can be affected by infection and drugs, wherever possible subjects for cytogenetic studies should not have taken drugs for 7 days prior to collection of blood for tests. Similarly, the mitotic index may be greatly reduced during the anergic phases of certain diseases (e.g. Hodgkin's disease, sarcoidosis, etc.) and, to a lesser degree, in normal individuals during the later stages of pregnancy. Preservative must not be added to blood samples for lymphocyte culture. Aseptic techniques are essential.

Blood samples should be tested without delay whenever possible. If absolutely necessary, they may be stored at 2°C to 8°C for no longer than 48 hours.

DIRECTIONS FOR USE[1,6]
Whole blood or separated leukocytes have been used for cytogenetic studies, but the former is the simplest and most widely used in routine studies. As with any cell culture procedure, optimal results are dependent on establishing adequate culture conditions. Serum additives should be screened prior to use to ensure the selection of batches free of inhibitory effects. Since the relative content of active PHA may vary slightly between different batches, it may be beneficial to test two concentrations of PHA.

A. Preparation of whole blood microcultures
1. Collect 5 - 20 mL of fresh blood in phenol-free heparin and mix by inversion.
2. Reconstitute PHA by adding 5 mL sterile distilled water using a sterile syringe.
3. Using aseptic technique, prepare required volume of microculture medium allowing 5 mL for each blood sample.
4. Dispense microculture medium to appropriately labeled sterile screw-capped bottles and aseptically add 0.1 mL reconstituted PHA. Immediately before culture, add 0.4 mL of heparinized blood using a sterile disposable syringe.
5. Incubate the culture at 37°C for 72 hours. Mix each bottle by inversion daily.

B. Harvesting the cultures
1. Remove culture bottles from the incubator 1.5 hours prior to harvesting.
2. Add 0.15 mL of a solution of Colcemid (25 µg/mL) to each culture.
3. Mix gently and return to the 37°C incubator.
4. Remove the cultures from the incubator and transfer to plastic graduated centrifuge tubes marked with sample details.
5. Centrifuge the cultures in a bench centrifuge at 500 x g for 5 minutes.
6. Remove most of the supernatant fluid and discard.
7. Resuspend the pellet in 6 to 8 mL of 75 mM potassium chloride solution pre-warmed to 37°C for 10 minutes.
8. Centrifuge as in step 5 and discard supernatant.
9. Using a Pasteur pipette slowly add 6 to 8 mL of freshly prepared acetic alcohol to the pellet while agitating constantly on a vortex mixer. Add the fixative dropwise at first, followed by a slow trickle to minimize cellular damage and formation of lumps.
10. Leave at 4°C for 10 minutes.
11. Centrifuge lightly, remove the supernatant fluid as before and slowly add an additional 5 mL of acetic alcohol to resuspend pellet.
12. Repeat step 11 twice more, re-suspending finally in 0.5 mL acetic alcohol. Use this cell suspension to prepare slides for examination. Care must be taken to avoid agitation of the cells.

C. Preparation of slides
1. Slides must be scrupulously clean. A suitable cleaning procedure is to soak the slides in chromic acid overnight, after which they should be washed in running water for at least half an hour and polished with a glass cloth.
2. Apply 1 or 2 drops of the resuspended cell preparation to the center of a glass slide and allow to spread.
3. Wipe excess fixative from the edges of the slide with filter paper.
4. At the first appearance of Newton's Rings, blow gently to speed final drying of the slide.
5. Stain with Giemsa or 2% acetic acid-orcein stain and mount.

LIMITATIONS OF THE PROCEDURE
The precise mechanism of lymphocyte transformation is not yet fully understood and unexplained technical failures sometimes occur. Serum factors, as yet unidentified, can cause interference but microbial contamination of the cell culture system remains the most frequent cause of failure. Hence, scrupulous attention to details of technique has to be given to lymphocyte culture systems if they are to be regularly successful. Optimal results are reliant on both the selection of culture conditions and reagents which support a good mitotic index. Hence, screening of serum additives prior to use, titration of PHA and a knowledge of the possible effect of patient history on lymphocyte response may be beneficial.

Purified Phytohemagglutinin is recommended for mitogenic studies which require precise mitogen dosage and the absence of inactive impurities.

CLINICAL APPLICATIONS
The modal human chromosome number is 46. Human chromosomes have been classified according to their length and the position of the centromere (Denver Classification[7]). Aberrations of chromosomal constitution have been associated with a number of congenital disorders such as Down syndrome (typically with an additional small autosome) and syndromes associated with indeterminate sexuality (Turner's syndrome, Klinefelter's syndrome and others, where the sex chromosomes are found to be abnormal). An acquired chromosomal abnormality can be detected in a proportion of the leukocytes in chronic myelocytic leukemia (the "Philadelphia" chromosome) and the progress of treatment may be assessed by following this marker. As the limit of tolerance is approached in radiation therapy, there is a marked increase in the proportion of cells with bizarre chromosomal constitution and the appearance of these cells has been used as a guide to dosage.

QUALITY CONTROL
Several factors including source of sample, culture conditions and selection of reagents can influence the result obtained. Users are advised to run each new batch of reagent in parallel with reference material of known suitable activity before adoption in routine use.