HiKaryoXL™ RPMI Medium

With L-Glutamine, FBS, PHA-P, Penicillin, Streptomycin and Sodium bicarbonate
1X Liquid Karyotyping Medium

Product Code: AL249A

Intended Use:

HiKaryoXL™ RPMI Medium is a karyotyping medium recommended for short term *in vitro* culture of peripheral blood lymphocytes for cytogenetic studies.

Principle and Interpretation:

Cytogenetic studies include metaphase and pro-metaphase studies carried out on lymphocytes to detect chromosomal aberrations associated with structural and numerical abnormalities. Lymphocytes come from normal peripheral blood and are mitotically inactive, hence have to be stimulated with a mitogen such as Phytohemagglutinin M (PHA-M) or Phytohemagglutinin P (PHA-P).

Phytohemagglutinin is a lectin extract from red kidney bean (Phaseolus vulgaris). PHA-P consists of two subunits, a leucoagglutinin (PHA-L) and an erythroagglutinin (PHA-E). PHA-P is a stronger blood agglutinin than PHA-M because of higher PHA-E (erythroagglutinin) activity. Therefore blood coagulation is commonly observed in media containing PHA-P. However blood coagulation does not in any way interfere with the karyotyping results.

AL249A is HiKaryoXL™ RPMI Medium composed of a basal medium RPMI 1640 and supplemented with L-Glutamine, FBS, PHA-P, Penicillin, Streptomycin and Sodium bicarbonate. It is a complete medium and does not require supplementation with any additional components.

Composition:

Ingredients	mg/L
INORGANIC SALTS	
Calcium nitrate tetrahydrate	100.000
Magnesium sulphate anhydrous	48.840
Potassium chloride	400.000
Sodium bicarbonate	2000.000
Sodium chloride	6000.000
Sodium phosphate dibasic anhydrous	800.000
AMINO ACIDS	
Glycine	10.000
L-Arginine hydrochloride	241.000
L-Asparagine	50.000
L-Aspartic acid	20.000
L-Cystine dihydrochloride	65.200
L-Glutamic acid	20.000
L-Glutamine	300.000
L-Histidine hydrochloride monohydrate	20.960
L-Hydroxyproline	20.000
L-Isoleucine	50.000
L-Leucine	50.000
L-Lysine hydrochloride	40.000
L-Methionine	15.000
L-Phenylalanine	15.000
L-Proline	20.000
L-Serine	30.000
L-Threonine	20.000
L-Tryptophan	5.000
L-Tyrosine disodium salt	28.830
L-Valine	20.000
VITAMINS	
Choline chloride	3.000
D-Biotin	0.200
D-Ca-Pantothenate	0.250
Folic acid	1.000
Niacinamide	1.000
Pyridoxine hydrochloride	1.000
Riboflavin	0.200
Thiamine hydrochloride	1.000
Vitamin B12	0.005
i-Inositol	35.000
p-Amino benzoic acid (PABA)	1.000
OTHERS	
D-Glucose	2000.000
Fetal Bovine Serum	Proprietary
Glutathione reduced	1.000
PHA-P	Proprietary
Penicillin	Proprietary
Streptomycin	Proprietary
Phenol red sodium salt	5.300

Type of Specimen:

Clinical samples – Blood

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling as per established guidelines[1, 2].

1. Disinfect the vacutainer by applying 70% isopropyl alcohol to the rubber stopper.
2. If using blood collection tube containing suitable anticoagulant (EDTA), disinfect the tube by applying 70% isopropyl alcohol.
3. Wait for 1 minute.
4. Palpate vein before disinfection of venipuncture site.
5. Starting at the center, swab the site concentrically with alcohol or chlorhexidine swabs.
6. Allow the disinfectant to dry.
   Note: Do not palpate the vein at this point without sterile gloves.
7. Collect the required volume of blood by venipuncture.
8. Mix gently by inverting tube 2 – 3times to avoid coagulation.
9. Sterilize the needle, syringe and other materials used for blood collection by autoclaving before discarding.

Warning and Precautions:

In Vitro Diagnostic Use only. Read the label before opening the container. Wear protective gloves / protective clothing / eye protection / face protection. Follow proper aseptic techniques while handling specimens and cultures. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety datasheets.

Directions:

1. Add freshly collected heparinized whole blood to 10ml of HiKaryoXL™ Medium in T-25cm^2 flasks as per the following recommendations:
   - Normal adults - 0.8ml
   - Infants and children- 0.6ml
   - Women (during pregnancy/ postpartum) - 1.0ml
2. Incubate the flasks at 37^{o}C and 5% CO_2 for 70-72 hours in upright position.
3. To determine optimum incubation time i.e. the peak mitotic index, collect samples at different time intervals between 48-72 hours.
   Note: Peak mitotic index is most commonly observed at 70-72 hrs.
4. Add 100µl of 10µg/ml of colchicine and incubate at 37^{o}C and 5% CO_2 for additional 2 hours.
   Note: Incubation time of less than 1 hour might result in reduced mitotic index.
5. Transfer the entire content of the flask to a sterile centrifuge tube and centrifuge at 800-1000rpm for 10 minutes.
6. Discard the supernatant and resuspend the pellet in 5ml of warm KCl solution (0.075M) and incubate in a water bath at 37^{o}C for 35 - 40 minutes.
   Note: Add KCl solution drop wise while agitating the cells.
7. Add 5ml of freshly prepared ice cold fixative (Acetic acid: methanol, 1:3 parts) and mix gently by inverting.
8. Centrifuge cells at 800-1000rpm for 10min.
9. Discard the supernatant and again add 5ml of freshly prepared ice-cold fixative (acetic acid: methanol, 1:3 parts) with constant mixing. Leave the cells at 4^{o}C for 10-15 min.
10. Centrifuge the cells at 1000rpm for 10 minutes.
11. Repeat step no. 10 and 11.
12. Discard the supernatant and resuspend the pellet in 0.5ml of fresh fixative. Store the tubes in refrigerator at 2 – 8^{o}C until use.
13. Clean the slides with mild detergent and wash thoroughly under tap water to make them grease free.
14. Place the clean slides in a beaker containing water such that they are completely immersed in water. Keep the beaker in a refrigerator at 2 – 8^{o}C and allow the slides to cool.
    Note: Steps 13 and 14 can be performed during incubation period of 2 hours with colchicine solution to save time.
15. Mix the cell suspension gently by pipetting up and down. Do not vortex.
16. Tilt the ice cold wet slide at 45^o angle and drop 50µl suspension at the bottom of slide with the help of micropipette in such a way that the suspension hits hard on the slide and then runs down surface.
17. Similarly drop 50µl suspension the center and 50µl at the top of the slide.
    Note: Ensure that the direction of dropping is from bottom to the top.
18. Allow the slides to air dry. Do not blow.
19. Fix them over a hot plate or a boiling water bath.
20. Stain the slides with required staining solution.

Materials required but not provided:

HiKaryoXL™ Colchicine Solution (TCL062) or
HiKaryoXL™ Colcemid® Solution (TCL074)
Potassium Chloride solution 0.075M (TCL040)
Methanol
Acetic Acid
Giemsa Stain (TCL083)

Limitations:

Not applicable.

Quality control:

Appearance
Orangish colored, clear solution

pH
7.00 -7.60

Osmolality in mOsm/Kg H2O
340.00 -380.00

Sterility
No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Cultural Response
The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by counting the metaphases.

Storage and shelf life:

Store at -20°C in a freezer that is not self-defrosting. Once thawed, the product is stable for about 30 days at 2 – 8ºC. Repeated freezing and thawing reduces mitogenic activity and should be avoided. Once thawed, the medium can be aliquoted into smaller volumes and frozen for future use.
Shelf life is 24 months.
Use before expiry date given on the product label.

Troubleshooting Tips:

Problem	Cause	Solution
No cell growth or very slow growth	Incubation temperature too high or too low	Check incubator temperature. It should be 37ºC ± 0.5ºC. Lower temperatures retard the growth rate. Higher temperatures usually result in cell death.
	CO_2 percentage in the incubator too high or too low	Check percentage of CO_2 inside the incubator. It should be 5 ± 0.5%
	Blood used for culture is not fresh	Always use fresh blood
No chromosomes or scattered chromosomes	Cells burst during harvest procedure	Ensure gentle addition of fixative and hypotonic solution
No metaphases	Harvesting not performed in exponential phase	Harvesting should be done between 70 – 72 hours
Chromosomes not well spread or non-uniform	Presence of cell aggregates	Disperse cell clumps before dropping the cell suspension on slide
		Drop the cell suspension on the slide from a height
	Non uniform drying of slide	Avoid blowing and always air dry the slide
	Slides not washed properly and not made grease-free	Ensure that the slides are clean and grease-free
Chromosomes contracted	Prolonged treatment with mitotic inhibitor	Repeat the procedure by treating the culture with mitotic inhibitor for recommended time

Disposal:

User must ensure safe disposal by autoclaving and / or incineration of used or unusable preparations of this product. Follow established laboratory procedures for disposing infectious materials. The materials that comes into contact with clinical samples must be decontaminated and disposed of in accordance with current laboratory techniques [1, 2].

References:

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.
2. Jorgensen, J. H., Pfaller, M.A., Carroll, K.C., Funke, G. Landry, M.L., Richter, S.S and Warnock., D.W. (2015), Manual of Clinical Microbiology, 11th Edition. Vol. 1.

IVD — In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

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