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Technical Datasheet

HiKaryoXL™ RPMI Medium

w/ L-Glutamine, FBS, PHA-M, Penicillin, Streptomycin and Sodium bicarbonate

Product Code: AL165A

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-M consists of two subunits, a leucoagglutinin (PHA-L) and an erythroagglutinin (PHA-E).

AL165A is HiKaryoXL™ RPMI Medium composed of a basal medium RPMI 1640 and supplemented with L-Glutamine, FBS, PHA-M, Gentamicin sulfate and Sodium bicarbonate. It is a complete medium and does not require supplementation with any additional component.

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-M	Proprietary
Gentamicin sulfate	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].

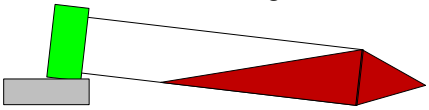
1. Disinfect the vacutainer by applying 70% isopropyl alcohol to the rubber stopper.
2. If using blood collection tube containing suitable anticoagulant (Heparin), disinfect the tube by applying 70% isopropyl alcohol.
3. Wait for 1 minute.
4. Palpate vein before disinfection of venipuncture site.
5.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

Warning and Precautions:

In Vitro Diagnostic Use only.
This Product Should be used by trained healthcare professionals/ lab technicians only.
Not to be Used for Self testing.
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.

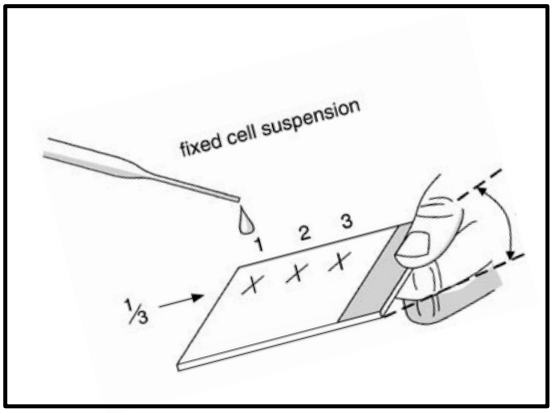
Directions

1. Add 500µl freshly collected heparinized whole blood to 5ml of HiKaryoXL™ Medium in a sterile 15ml conical bottom centrifuge tube.
2. Loosen the cap of tube by one thread and incubate at 37°C and 5% CO_2 for 70 - 72 hours in horizontal position as shown in the figure below.



Note: Alternatively, the tubes can be incubated in a non-CO_2 incubator. Absence of CO_2 does not affect the mitotic count.

3. Add 250µl of 10µg/ml of Colchicine / Colcemid® (TCL062 / TCL074 / TCL133) and incubate at 37°C for additional 20 minutes.
   Note: Incubation of 2 hours gives higher mitotic count than 20 minutes. Users are advised to decide incubation time as per their need and convenience.
4. After incubation with colchicine, centrifuge the tubes at 2000rpm for 4 minutes.
5. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
6. Add 5ml 0.075M Potassium chloride solution (TCL040) and incubate at room temperature for 7 minutes keeping the tubes in an upright position. Mix by inverting.
7. Centrifuge the tubes at 2000rpm for 4 minutes.
8. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
9. Add 5ml of freshly prepared ice cold fixative drop by drop (Acetic acid: methanol, 1:3 parts) and mix gently by inverting.
   Note: Addition of fixative for the first time may create turbulence which in turn may lead to cell breakage and irreversible clumping. Hence, fixative addition for the first time should be done dropwise and slowly.
10. Repeat steps 7, 8 and 9 two more times.
11. Resuspend the pellet in 0.5ml of fresh fixative and store them at -20°C till slide preparation.
12. Clean the slides with mild detergent and wash thoroughly under tap water to make them grease free.
13. 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°C and allow the slides to cool. *Note: Steps 12 and 13 can be performed during incubation period of 2-4 hours with colchicine solution to save time.*
14. Mix the cell suspension gently by pipetting up and down. DO NOT vortex.
15. Hold the ice cold wet slide at 45° 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. Refer the figure mentioned below.



16. 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.
17. Allow the slides to air dry. DO NOT blow.

18. Heat fix the slides by holding them over a hot plate for 10 – 12 seconds, with chromosome spreads facing up.
19. Stain the slides with required staining solution.

Materials required but not provided:

HiKaryoXL™ Colchicine Solution (TCL062) or
HiKaryoXL™ Colcemid® Solution (TCL074)
HiKaryoXL™ Colcemid® Solution (TCL133)
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 H_2O
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 -30ºC to -10ºC 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.
Colcemid is a registered trademark of Ciba-Giegy Corp.

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 come 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.

In vitro diagnostic medical device

CE Marking

Consult instructions for use

Do not use if package is damaged

Reg. Off : HiMedia Laboratories Pvt. Ltd., Plot No. C40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane (West) 400604, Maharashtra, India.
Works : B-4-5-6 / MIDC, Palkhed, Dindori, Nashik- 422202 Maharashtra, India
www.himedialabs.com

CE Partner 4U, Esdoornlaan 13, 3951 DB Maarn The Netherlands,www.cepartner 4u.eu

Contains biological material of animal origin

Sterilized using aseptic processing techniques

Do not re-use