IV-Cell™ Cytogenetics Media

RUO Product Information Packet

Precipio, Inc.
4 Science Park
New Haven, CT 06511, USA
Tel: +1.203.787.7888
support@precipiodx.com

www.precipiodx.com

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1. Product Overview

IV-Cell universal culture media is a Research-use only (RUO), ready-to-use, fully supplemented medium developed specifically to support bone marrow and peripheral blood cell culture for *in vitro* cytogenetic analysis of hematological disease.

IV-Cell is a proprietary culture media that enables simultaneous culturing of all four (4) hematopoietic cell lineages to solve the problem of selective culturing. IV-Cell has been optimized for bone marrow and peripheral blood culturing that includes all the necessary components to stimulate the growth of:

- Myeloid cells
- T-Lymphocytes
- B-Lymphocytes
- Plasma Cells

Product	IV-Cell Culture Media
Catalog Number	7000-IVC
Amount	500 mL
Storage	-20°C (protect from light)
Shelf Life*	12 months

* *Shelf Life is determined from date of manufacturing*
Do not use beyond the expiration date

Media contents:
- Serum protein
- Media base
- Growth hormones
- Enhancers
- B-Cell mitogens
- T-Cell mitogens
- Plasma-Cell mitogens

Key Product Advantages:

- ✓ Clinical benefit – culturing all four cell lineages simultaneously
- ✓ All-in-one media requires no chemical mixing
- ✓ Reduced number of SKUs simplifies inventory management, product ordering, and QC testing
- ✓ Improved performance of plasma cell stimulation
- ✓ Higher band resolution reduces karyotyping tech-time

2. Intended Use

For in vitro diagnostic use only. Research Use Only (RUO) Product.

Important Information

- IV-Cell must be stored frozen at -20°C and protected from direct light to avoid stimulation of the mitogens.
- When the lab is ready to use a bottle, let frozen media thaw completely at room temperature (protected from light) before use.
- Expected thaw time is ~30-45 mins (no longer than 1 hour at room temperature).
- Upon completion of thawing, media must be used immediately, within 1.5 hour total timeframe from removal from freezer.
- If the anticipated volume does not require use of entire contents of the bottle, remaining media can be either:
  - stored in refrigeration, and consumed within 24 hours.
  - Aliquoted in 10ml vials and re-frozen.

THE MEDIA CAN BE RE-FROZEN ONLY ONCE AFTER THAWING AND ALIQUOTING, BUT CAN NOT BE FROZEN AGAIN

Bone marrow or leukemic peripheral blood received should be set-up in duplicates according to the indication for that particular study requested to provide backup cultures in the event of failures due to contamination, technical error and other problems, as well as providing the best opportunity to verify true mosaicism.

Aliquot Instructions

To aliquot IV-Cell™ 500ml bottle, please follow the steps below:

- Thaw IV-Cell™ 500 ml bottle completely (at room temperature, while avoiding direct light exposure).
- Prepare the sterile Biological hood by wiping the surface using 70% Ethyl alcohol.
- Wipe the external surface of the IV-Cell™ 500 ml bottle surface using 70% Ethyl alcohol.
- Prepare a sufficient number of 15ml sterile tubes necessary to aliquot the media.
- Using transfer pipette, aliquot 10ml of IV-Cell™ media to the 15ml sterile tube.
- Once the aliquot is completed, close the 15ml sterile tube tightly.
- The new aliquots should be stored protected from direct light at -20^{0}C.

REMINDER: after aliquoting, the media can be re-frozen once **ONLY**.

Safety Information

Read the material Safety Data Sheets (SDS) and follow recommended handling instructions. Wear appropriate protective personal protective equipment (eyewear, clothing & gloves). Handle in accordance with established bio-safety practices.
The material safety data sheets can be downloaded from the following link: www.precipiodx.com/ivcell.html

3. Instructions for Use

1. Cell Count using Hemocytometer

1.1. Prepare TRYPAN BLUE solution by mixing 95mL dH2O, 3mL acetic acid and 2mL Wright's stain
1.2. Make a 1:100 Dilution of the specimen by adding 5µl of specimen to 0.5 mL of 3% Acetic acid in an Eppendorf tube
1.3. Vortex well
1.4. Apply 5µl of the mixture to the Hemocytometer
1.5. Wait for a few minutes for cells to settle down on the slide
1.6. Count cells present in the square labeled **"C"** as shown in the picture
1.7. In case of low cell count, calculate an average cell count:
   - Calculate the sum of the cells present in all four quadrants labeled "**B**"
   - Divide the sum by 4 to get the average
1.8. Use the table below to identify the required specimen volume to be used

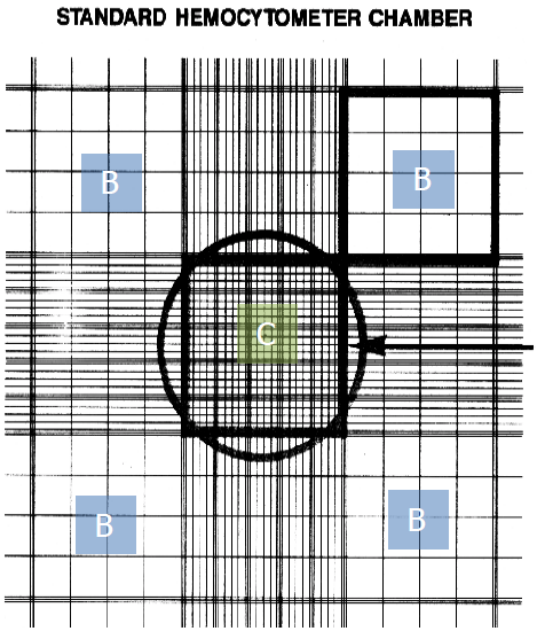


Table 1: Specimen Volume Requirements

Cell Count (1:100 Dilution)	Specimen volume (µL)
10	1200
20	700
30	500
40	300
50	300
60	200
70	200
80	200
90	150
>= 100	150

2. Culture Set-up

2.1. Thaw IV-Cell culture media
   2.1.1. At room temperature (protected from light) completely before use
   2.1.2. Avoid thawing at 37°C
   2.1.3. Once thawed, the unopened bottle of IV-Cell can be stored at 2-8°C (protected from light) for up to 24 hrs.
   2.1.4. For optimal performance, avoid re-freezing and prolonged exposure to light
2.2. Once media is thawed and ready to use, keep stored inside the bio-hood to avoid contamination
2.3. Calculate sample cell count using 1:100 dilution
2.4. Set up all cultures in conical tubes using 10 mL IV-Cell media and specimen volumes per table in section 1.8 above
2.5. Follow table below for incubation times per cell type of interest

Table 2: Culture Incubation Periods

Cell Type	Incubation Period (Culture 1)	Incubation Period (Culture 2)
Myeloid Cells	18-24 hours	36-48 hours
T-Lymphocytes	24 hours	72 hours
B-Lymphocytes	24 hours	96 hours
Plasma Cells	24 hours	96-120 hours

3. Culture Harvesting

3.1. Have sufficient amount of hypotonic solution (KCl) at 37°C for the cultures to be harvested that day
3.2. Prepare fresh fixative (3 parts Methanol : 1 part Acetic Acid) at room temperature for the cultures to be harvested that day
3.3. Take out Ethidium Bromide and Colcemid from the refrigerator, and keep under the bio-hood.
3.4. Add 80uL EB (Ethidium bromide) to a 10mL culture, mix well by pipetting up and down without introducing bubbles.
3.5. Incubate at 37°C for 45 minutes
3.6. Add 100uL of Colcemid to the 10mL culture, mix well by pipetting up and down without introducing bubbles. Incubate at 37°C for 30 min
3.7. Transfer the culture from culture flasks to appropriately labeled 15 mL tube
3.8. Centrifuge for 9 min at 1200 rpm
3.9. Aspirate the supernatant carefully, leaving around 0.5 mL supernatant above the pellet
3.10. Gently mix the pellet then add hypotonic solution (previously warmed at 37°C) up to 12 ml.
3.11. Gently mix by inverting three times and then incubate at 37°C for 18 min
3.12. Remove from incubator and add 2 mL freshly prepared fixative slowly against the wall of the tube (two layers visible)
3.13. Invert to mix and centrifuge for 9 min at 1200 rpm
3.14. Aspirate the supernatant carefully leaving around 0.5 mL supernatant above the pellet. Gently mix the pellet by tapping a finger along the tip of the tube. Add fixative up to 10mL and gently mix with transfer pipette. Centrifuge for 9 minutes at 1200 rpm
3.15. Aspirate the supernatant carefully leaving around 0.5 mL supernatant above the pellet, gently mix the pellet by tapping a finger along the tip of the tube. Add fixative up to 5ml (or 3ml if pellet is very small) and gently mix by tapping a finger along the tip of the tube. Centrifuge for 9 minutes at 1200 rpm
3.16. If supernatant is not clear, then proceed with an additional 3ml wash by repeating the previous step using 3ml of fixative to wash
3.17. It is recommended to leave the pellet in the refrigerator for 30 min before dropping for higher quality banding

4. Slide Preparation

4.1. Centrifuge the cell suspension for 9 minutes at 1200 rpm and aspirate the suspension as before and then re-suspend pellet in a small volume of fixative (0.5 - 1mL). The pellet should be white and the suspension should appear cloudy after re-suspension
4.1.1. *Evaluate solution made:* More fixative can be added if too concentrated or spun down & re-suspend in smaller volume if too diluted. Use a new transfer pipette for each culture tube.
4.2. Drop slides from one case at the time
4.3. Use a clean slide from the slide box, run the slide under room temperature running water
4.4. While holding the slide at a 45-degree angle (4 o'clock) drop ~3 drops of the cell suspension on to the slide
4.5. Place the slide on a water bath rack for 4 minutes until slide is dry
4.6. Remove and wipe the back and sides of each slide
4.7. Prepare 3-4 slides per culture or as needed
4.8. Place the slides in the oven at 90°C for 30 minutes.

5. Slide Staining

5.1. Set up 6 staining jars and proceed with staining slides per the table below
5.2. Let slides air dry at an angle or use bibulous paper
5.3. Store slides at room temperature

Table 3: Slide Staining Procedure

Jar #	Contents	Procedure
Jar 1	Mixture of 40 mL Balanced Salt pH 7.0 & 5 mL Trypsin	Submerge slides for 50 seconds
Jar 2	40 mL Balanced Salt pH 7.0	Dip slides twice
Jar 3	40 mL Balanced Salt pH 7.0	Dip slides twice
Jar 4	Mixture of 47 mL Gurr Buffer pH 6.8 & 3 mL Giemsa	Submerge slides for 1 min & 10 sec
Jar 5	40 mL Distilled water	Dip slides twice
Jar 6	40 mL Distilled water	Dip slides twice

4. Validation Studies

A. Internal (Precipio) Validation Study

An internal side-by-side comparison study of IV-Cell compared to MarrowMax™ and a home brewed media was conducted by Precipio. The following table depicts the results summary:

Case #	Indication	Number of Cultures		Abnormality Detected	
		MX	IV-Cell	MX	IV-Cell
1	MDS	C1 + C2	C1 + C2	Normal	Normal
2	Thrompocytosis	C1 + C2	C1 + C2	Normal	Normal
3	MGUS/Multiple Myeloma	C1 + CB	C1 + CB	Normal	Normal
4	MDS	C1 + C2	C1 + C2	Abnormal	Abnormal
5	CML	C1 + C2	C1 + C2	Normal	Normal
6	CLL	C1 + CB	C1 + CB	Abnormal	Abnormal
7	Anemia	C1 + C2	C1 + C2	Normal	Normal
8	MDS	C1 + C2	C1 + C2	Normal	Normal
9	Anemia	C1 + C2	C1 + C2	Normal	Normal
10	MGUS/Multiple Myeloma	C1 + CB	C1 + CB	Abnormal	Abnormal

Legend	
MARROWMAX™	MX
IV-Cell Mecium	IV-Cell
24 hr un-stimulated culture	C1
48 hr un-stimulated culture	C2
96 hr stimulated culture	CB
Lipopolysaccharides	LPS
Interleukin-2	IL2

B. External Laboratory comparative study:

A side-by-side comparison study of IV-Cell and MarrowMax™ was conducted by an external US CLIA and CAP certified laboratory.

The following table depicts the results summary:

	Culture	Media	Harvest Method	Media Vol (ml)	Mitotic Index (MI)	Quality	Band Resolution
Myeloid							
Patient 1	24EB MM	Marrow Max	Current	5			
	24EB	Precipio	Current	5			
	24EB IVC	Precipio	Precipio	5			
	24EB IVC10	Precipio	Precipio	10			
Patient 2	48EB MM	Marrow Max	Current	5	Very Poor	Optimal	400
	48EB	Precipio	Current	5	Poor	Sub-Optimal	400
	48EB IVC	Precipio	Precipio	5	Poor	Optimal	some ≥400
	48EB IVC10	Precipio	Precipio	10	Fair	Optimal	some ≥400
Lymphoid							
Patient 3	72IL2 MM	Marrow Max	Current	5	Good	Acceptable	400
	72IL2	Precipio	Current	5	very overspread	very overspread	very overspread
	72IL2 IVC	Precipio	Precipio	5	Good	Acceptable	some ≥400
	72IL2 IVC10	Precipio	Precipio	10	Very Good	Optimal	550
Patient 4	72IL2 MM	Marrow Max	Current	5	Fair	Acceptable	400
	72IL2	Precipio	Current	5	Very Poor	Sub-Optimal	400
	72IL2 IVC	Precipio	Precipio	5	Fair	Acceptable	some ≥400
	72IL2 IVC10	Precipio	Precipio	10	Good	Optimal	550
Patient 5	72PHA MM	Marrow Max	Current	5	Fair	Optimal	550
	72PHA	Precipio	Current	5	Fair	Optimal	550
	72PHA IVC	Precipio	Precipio	5	Good	Optimal	550
	72PHA IVC10	Precipio	Precipio	10	Very Good	Optimal	550
Plasma Cell							
Patient 6	120IL4 MM	Marrow Max	Current	5	Fair	Acceptable	400
	120IL4	Precipio	Current	5	Very Good	Optimal	550
	96IL4 IVC	Precipio	Precipio	5	Very Good	Optimal	550
	96IL4 IVC10	Precipio	Precipio	10	Very Good	Optimal	550
Patient 7	120IL4 MM	Marrow Max	Current	5	Very Poor (~2-3 mets)	Sub-Optimal	≤400
	120IL4	Precipio	Current	5	Very Poor (~4-6 mets)	Sub-Optimal	400
	96IL4 IVC	Precipio	Precipio	5	Fair	Acceptable	400
	96IL4 IVC10	Precipio	Precipio	10	Very Good	Optimal	550

Mitotic index key:	Metaphase quality key:
Good: 9 metaphases	**Optimal:** >95% intact, cytoplasm free, good length
Fair: 6-8 metaphases	**Acceptable:** 70-90% intact spreads, moderate cytoplasm, varying lengths
Poor: 3-5 metaphases	**Sub-optimal:** >85% tight spreads, heavy cytoplasm, very short, indistinguishable chromosomes
Very poor: 1-2 metaphases	
NMS: no metaphase seen	

5. Performance Results (Internal)

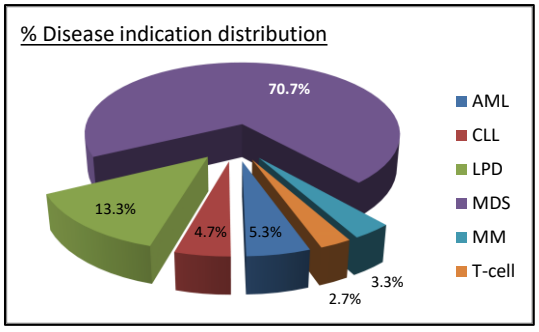
Overview:
IV-Cell Media was first used clinically at Precipio's laboratory in 04/2017. The following data presents Precipio's internal results from the application of IV-Cell media to Karyotyping cases within Precipio's department of cytogenetics.

Method:
150 Bone Marrow Biopsy cases were randomly selected where Karyotyping was conducted. The results depict the disease state distribution and the banding resolution received by using IV-Cell media.

Results:

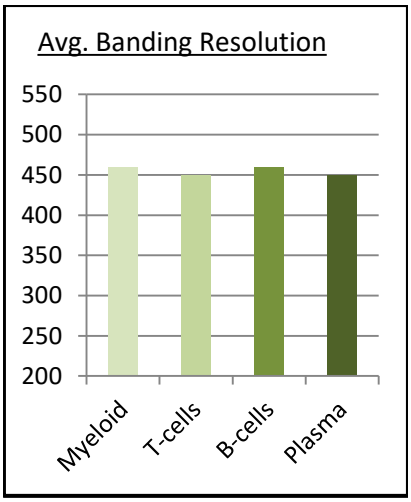
As shown in the images on this page, IV-Cell media provided consistently high results as measured by the banding resolution. Average band resolution is 460, with some chromosomes reaching banding resolution of above 500.

The images below represent a side-by-side demonstration of the karyotypes using an on-market media vs. Precipio's IV-Cell media.

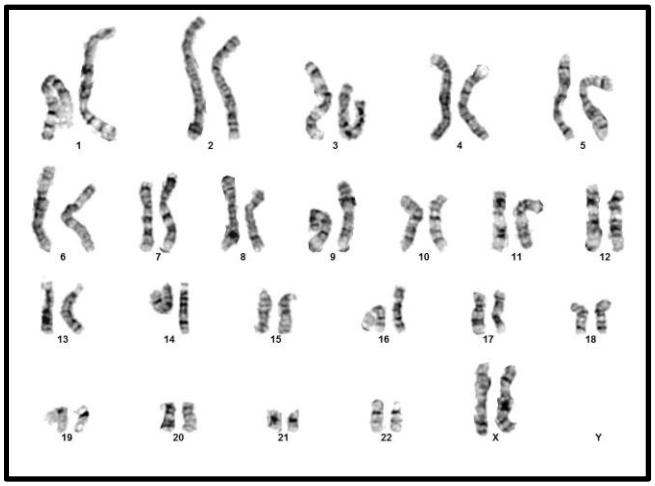


Summary table:	
Number of cases:	N=150
Avg. Banding Resolutior	460

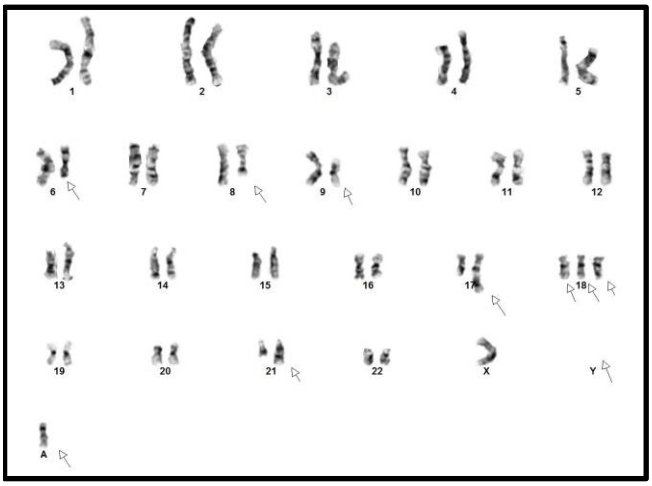
Disease	#	%
AML	8	5.3%
CLL	7	4.7%
LPD	20	13.3%
MDS	106	70.7%
MM	5	3.3%
T-cell	4	2.7%
	150	100.0%



High Resolution Normal Karyotype (550 band resolution):



High Resolution abnormal karyotype (500 band resolution):



6. Explanation of Symbols and Warnings

			
Caution, consult accompanying documents	*In vitro* diagnostic medical device	Sterilized using aseptic processing techniques	Keep away from light
			
Use By:	Catalog number	Manufacturer	Batch Code
			
European Community	Consult instructions for use	Temperature Limitation	

Each manufactured lot of IV-Cell™ is performance-tested on primary normal bone marrow cells to ensure product performance for in vitro diagnostic use for this application.

Each clinician/scientist must make an independent judgment on whether this medium is suitable for use in In Vitro Diagnostic applications conducted in their laboratory. Precipio does not guarantee the successful outcome of any diagnostic testing based solely on the use of IV-Cell medium. Precipio contribution to these procedures is simply at the step of providing a culture or handling medium for these procedures.

Technical Support Contact Information

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4 Science Park
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TechSupport@precipiodx.com
www.precipiodx.com

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