LEUKOSTOR™

PRESERVATION OF LEUKAPHERESIS

LS-10; LS-30; LS-70

Atelerix Handbook Series

Version LS04;0.1.1

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1. COMPONENTS

1.1. KIT CONTENTS

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	APHERESIS VOLUME TO ADD
LS-10S-03	GelBase Beads	3 Tubes	10 mL	-
	Gel A (5x)	3 Tubes	2.5 mL	10 mL
	Dissolution Buffer	3 Tubes	20 mL	-
	MACS SmartStrainers	6	-	-
LS-10S-036	GelBase Beads	6 Tubes	10 mL	-
	Gel A (5x)	6 Tubes	2.5 mL	10 mL
	Dissolution Buffer	6 Tubes	20 mL	-
	MACS SmartStrainers	12	-	-
LS-10S-12	GelBase Beads	12 Tubes	10 mL	-
	Gel A (5x)	12 Tubes	2.5 mL	10 mL
	Dissolution Buffer	12 Tubes	20 mL	-
	MACS SmartStrainers	24	-	-
LS-10S-24	GelBase Beads	24 Tubes	10 mL	-
	Gel A (5x)	24 Tubes	2.5 mL	10 mL
	Dissolution Buffer	24 Tubes	20 mL	-
	MACS SmartStrainers	48	-	-
LS-10S-50	GelBase Beads	50 Tubes	10 mL	-
	Gel A (5x)	50 Tubes	2.5 mL	10 mL
	Dissolution Buffer	50 Tubes	20 mL	-
	MACS SmartStrainers	100	-	-
LS-30S-03	GelBase Beads	3 Tubes	30 mL	-
	Bag A (5x Gel A)	3 Tubes	7.5 mL	30 mL
	Bag C (Dissolution Buffer)	3 Tubes	45 mL	-
	Blood Administration Set	3	-	-
	Line Clamps	9	-	-
LS-30S-06	GelBase Beads	6 Tubes	30 mL	-
	Gel A (5x)	6 Tubes	7.5 mL	30 mL
	Bag C (Dissolution Buffer)	6 Tubes	45 mL	-
	Blood Administration Set	6	-	-
	Line Clamps	18	-	-
LS-30S-12	GelBase Beads	12 Tubes	30 mL	-
	Gel A (5x)	12 Tubes	7.5 mL	30 mL
	Bag C (Dissolution Buffer)	12 Tubes	45 mL	-
	Blood Administration Set	12	-	-
	Line Clamps	36	-	-

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	APHERESIS VOLUME TO ADD
LS-30S-24	GelBase Beads	24 Tubes	30 mL	-
	Gel A (5x)	24 Tubes	7.5 mL	30 mL
	Bag C (Dissolution Buffer)	24 Tubes	45 mL	-
	Blood Administration Set	24	-	-
	Line Clamps	72	-	-
LS-30S-50	GelBase Beads	50 Tubes	30 mL	-
	Gel A (5x)	50 Tubes	7.5 mL	30 mL
	Bag C (Dissolution Buffer)	50 Tubes	45 mL	-
	Blood Administration Set	50	-	-
	Line Clamps	150	-	-
LS-70S-03	GelBase Beads	50 Tubes	70 mL	-
	Gel A (5x)	50 Tubes	17.5 mL	70 mL
	Bag C (Dissolution Buffer)	50 Tubes	105 mL	-
	Blood Administration Set	3	-	-
	Line Clamps	9	-	-
LS-70S-06	GelBase Beads	50 Tubes	70 mL	-
	Gel A (5x)	50 Tubes	17.5 mL	70 mL
	Bag C (Dissolution Buffer)	50 Tubes	105 mL	-
	Blood Administration Set	6	-	-
	Line Clamps	18	-	-
LS-70S-12	GelBase Beads	50 Tubes	70 mL	-
	Gel A (5x)	50 Tubes	17.5 mL	70 mL
	Bag C (Dissolution Buffer)	50 Tubes	105 mL	-
	Blood Administration Set	12	-	-
	Line Clamps	36	-	-
LS-70S-24	GelBase Beads	50 Tubes	70 mL	-
	Gel A (5x)	50 Tubes	17.5 mL	70 mL
	Bag C (Dissolution Buffer)	50 Tubes	105 mL	-
	Blood Administration Set	24	-	-
	Line Clamps	72	-	-
LS-70S-50	GelBase Beads	50 Tubes	70 mL	-
	Gel A (5x)	50 Tubes	17.5 mL	70 mL
	Bag C (Dissolution Buffer)	50 Tubes	105 mL	-
	Blood Administration Set	50	-	-
	Line Clamps	150	-	-

Note: Remove components from 2-8°C for at least 20 minutes before use.

1.2. COMPONENTS TO BE SUPPLIED BY THE USER

• Leukopak wash buffer [recommended RPMI 1640 + 2% FBS + 1mM EDTA] • Cell culture medium • Blood bag tube welder (LS-30, LS-70: optional) • Laboratory rocker • Class II microbiological safety cabinet (optional) • 500 ml polystyrene storage boxes (optional) • 50 ml centrifuge tubes • 50 ml centrifuge racks • 10 – 50 ml serological pipettes and pipette controller • Centrifuge • Category B biological substance bag • Absorbent pads • Shipping container (capable of holding at the appropriate temperature, see section 4.4) • Apheresis Material!!

1.3. BEFORE YOU BEGIN USING LEUKOSTOR™

1. Ensure LeukoStor™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.

2. Read the troubleshooting guide on page 15 to see our list of frequently asked questions. For any further queries, please email us at Sales@atelerix.co.uk.

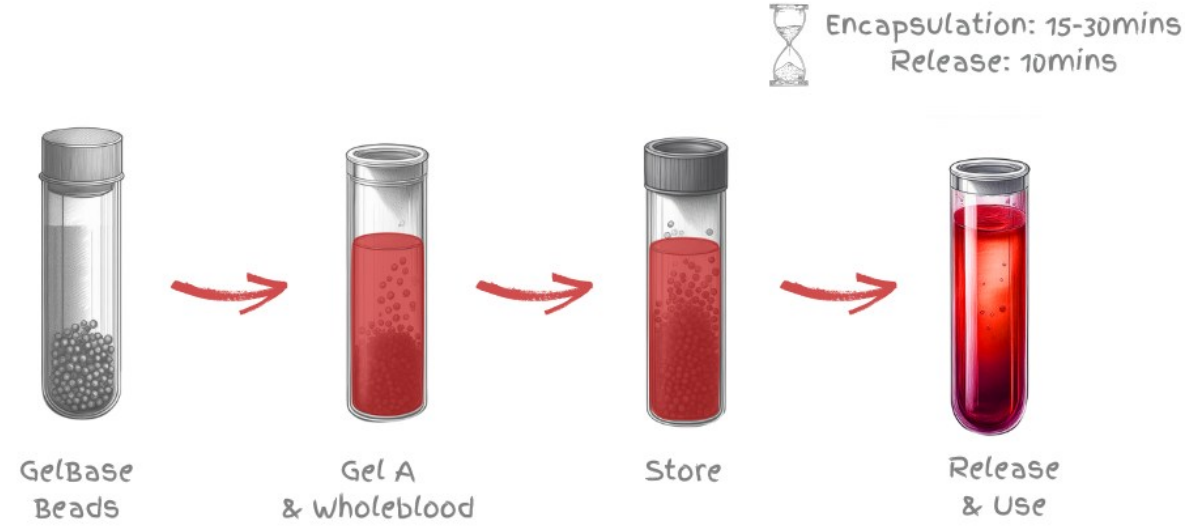
3. LeukoStor™ is intended for use solely in accordance with this protocol using the components provided within the kit.

2. SAMPLE SIZE GUIDE

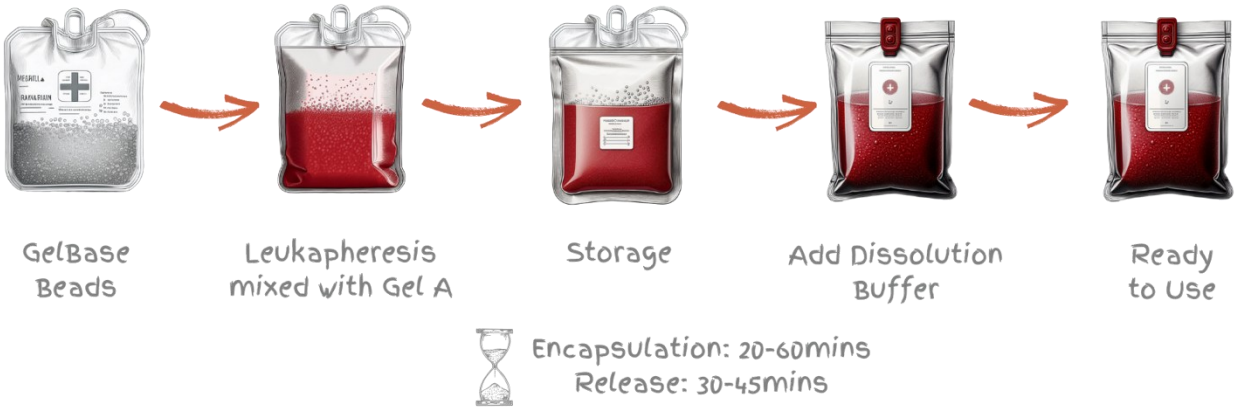
Product Name	Volume	Leukopak Size	Total Cell Volume per Leukopak Size
LeukoStor™-10 (LS-10)	10 mL	1/30 Leukopak	2-4 x 10^8 Cells
LeukoStor™-30 (LS-30)	30 mL	1/8 Leukopak	0.5-1 x 10^9 Cells
LeukoStor™-70 (LS-70)	70 mL	1/4 Leukopak	1-2x 10^9 Cells

3. PROTOCOL OVERVIEW

3.1. LEUKOSTOR™-10 (LS-10)



3.2. LEUKOSTOR™-30 (LS-30) AND LEUKOSTOR™-70 (LS-70)



4. LEUKOSTOR-10™ (LS-10)

4.1. GELATION

Stage 1

1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes.

2. Prepare the leukapheresis normally. Leukapheresis material should be transferred to a suitable sterile container.

3. Transfer 10 mL leukapheresis material to the tube containing Gel A and mix thoroughly using a 10 mL serological pipette.

Stage 2

4. Transfer 12.5 mL of Gel A / leukapheresis mixture into the GelBase Beads[1] using a 10 mL or 25 mL serological pipette.

5. Place the cap back on the tube and gently invert the **gel / bead** mixture several times until the beads are evenly distributed throughout the gel. Gently flick the tube to settle contents, ensuring a tight seal (the gel will cure *in situ* within approximately 20 minutes, sample is ready to ship after 1 hour).

Stage 3

6. Prepare sample for shipping. Place inside a category B biological substance bag with some absorbent pads.

7. Store away from light in a polystyrene box at the recommended temperature for the cell type encapsulation (see section 4.2). Alternatively, for the most up to date recommendations on storage temperatures and times per immune cell subpopulation, visit our Compatibility section on our website or contact sales@atelerix.co.uk.

[1]Use the LeukoStor Vial containing beads provided for encapsulation, storage, and release.

4.2. CELL DENSITY AND LOADING GUIDE

The below recommendations yield the highest viable recovery of the indicated cell type. To preserve all leukocyte populations, we recommend storing at 25°C.

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
T-Cells	25°C	5 Days
NK-Cells	25°C	5 Days
B-Cells	2-8°C	5 Days
Monocytes	2-8°C	5 Days

If you cannot find any recommendations for your cell type, please contact Sales@atelerix.co.uk.

4.3. RELEASE

Stage 1

1. Remove Dissolution Buffer from the refrigerated storage and allow to equilibrate at room temperature for at least 30 minutes.

2. Remove gelled apheresis material from its shipping container and transfer to a Class II Microbiological Safety Cabinet.

3. Transfer the gelled leukapheresis material into a 50 mL centrifuge tube by inversion and gentle tapping.

4. Add 20 mL Dissolution Buffer to the first tube to wash before transferring to the tube containing gelled aphesis material. Replace the cap, ensuring a tight seal.

5. Place on a rocker set to 40rpm for 30 minutes to dissolve the gel encapsulating leukapheresis.

Stage 2

6. Place the tube containing dissolved apheresis material in a Class II Microbiological Safety Cabinet.

7. Prepare 2 x 50 mL centrifuge tubes placing cell strainers onto the uncapped tubes.

8. Remove cap and decant the contents into cell strainers. Material in the cell strainers can be stirred using a 3 mL Pasteur pipette avoiding touching the membrane. This will encourage the material to flow through the strainer.

9. Once all apheresis has been strained, wash GelBASE beads in the strainer with 10 mL leukopak wash buffer using a 10 mL serological pipette.

10. Dispose of cell strainers containing GelBASE beads via the appropriate route.

11. Sediment washed apheresis material using a centrifuge set to 350 x g for 10 minutes with soft deceleration.

12. Resuspend leukapheresis material with a suitable volume of cell culture medium ready for downstream processes

4.4. SHIPPING YOUR SAMPLE

Use appropriate controlled temperature packaging[2] when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

[2]For best cell recovery upon arrival, we recommend using the ICECATCH Solid Ambient or Cool Shipping Boxes.

Find out more & shop at https://www.atelerix.co.uk/pages/variants-collection-page-accessories

5. LEUKOSTOR-30™ (LS-30)

5.1. GELATION

Stage 1

1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes.

2. Prepare the leukopak normally. The attached tubing should be sealed and clipped at a point close to the bag and any used ports should also be sealed.

3. Remove the protective tab from an unused port on the leukopak.

4. Remove the protective cover from the spike attached to Bag A. Spike the uncovered port on the leukopak and ensure the spike is correctly inserted*.
*alternatively, if the leukopak has an existing transfer line with a spike, this can be used to transfer the material to Bag A via an unused port.

5. Mobilise the contents of the leukopak by gently massaging for 10s and then drain the material into Bag A via the spike connection and seal the transfer tube with a clip.

6. Optionally seal the tubing connecting the two bags and detach them from each other.

7. Mix the contents of Bag A by gently massaging the material for 30s, whilst inverting the bag to ensure the contents are fully mixed.

Stage 2

8. Ensure that the beads in Bag B are well separated by gently massaging the beads.

9. Remove the protective tab from an unused port on Bag A.

10. Remove the protective cover from the spike attached to Bag B. Spike the uncovered port on Bag A and ensure the spike is correctly inserted.

11. Mobilise the contents of Bag A by gently massaging for 10s, and then remove the clip from the line connecting Bag A to Bag B. Drain the material into Bag B via the spike connection, and then replace the clip at Bag B.

12. Seal the tubing connecting the two bags and then detach them from each other.

13. Mix the contents of Bag B by placing on a rocker set to 30rpm for 15 minutes.

14. Lay Bag B flat on a bench and leave for 1 hour at room temperature, avoiding agitation.

15. Bag B alone needs to be shipped; the other materials can be discarded in the appropriate manner.

Stage 3

16. Prepare Bag B for shipping. Place Bag B inside a category B biological substance bag with some absorbent pads.

17. Seal the bag and then lay it flat inside a shipping container along with Bag C (Dissolution Buffer).

18. Store away from light in a polystyrene box at the recommended temperature for the cell type encapsulation (see section 5.2). Alternatively, for the most up to date recommendations on storage temperatures and times per immune cell subpopulation, visit our Compatibility section on our website or contact sales@atelerix.co.uk.

Use the LeukoStor Bag containing beads provided for encapsulation, storage, and release.

5.2. CELL DENSITY AND LOADING GUIDE

The below recommendations yield the highest viable recovery of the indicated cell type. To preserve all leukocyte populations, we recommend storing at 25°C.

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
T-Cells	25°C	5 Days
NK-Cells	25°C	5 Days
B-Cells	2-8°C	5 Days
Monocytes	2-8°C	5 Days

If you cannot find any recommendations for your cell type, please contact Sales@atelerix.co.uk.



5.3. RELEASE

Stage 1

1. Remove Bag B and Bag C (Dissolution Buffer) from the shipping container.

2. Remove the protective tab from an unused port on Bag B.

3. Remove the protective cover from the spike attached to Bag C. Spike the unused port on Bag B and ensure the spike is correctly inserted.

4. Remove the clip on Bag C before draining the dissolution buffer from Bag C into Bag B via the spike connection and then replace the clip close to Bag B.

Stage 2

5. Remove Bag B from the rocker and place in a Class II Microbiological Safety Cabinet.

6. Take the Blood Administration Set from its packaging and ensure the tube is clamped, then remove the protective cover from its spike.

7. Remove existing spike from the port in Bag B and insert the spike from the Blood Administration Set.

8. Hang Bag B at a height.

9. Remove cap from the Blood Administration Set line.

10. Unclamp the line and allow to strain into collection vessels ensuring enough space remains in the vessels for the addition of an equal volume of wash buffer (i.e. if draining into 50 mL centrifuge tubes, add 25 mL dissolved leukopak material).

11. Add an equal volume of wash buffer to the dissolved leukopak material (i.e. if draining into 50 mL centrifuge tubes, add 25 mL wash buffer).

12. Washed leukopak material can now be used directly for downstream processing or sedimented to retrieve leukocytes*. ***Sedimentation using a centrifuge set to 350 x g for 10 minutes with soft deceleration is recommended.**

5.4. SHIPPING YOUR SAMPLE

Use appropriate controlled temperature packaging[2] when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

[2]For best cell recovery upon arrival, we recommend using the ICECATCH Solid Ambient or Cool Shipping Boxes.

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6. LEUKOSTOR-70™ (LS-70)

6.1. GELATION

Stage 1

1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes.

2. Prepare the leukopak normally. The attached tubing should be sealed and clipped at a point close to the bag and any used ports should also be sealed.

3. Remove the protective tab from an unused port on the leukopak.

4. Remove the protective cover from the spike attached to Bag A. Spike the uncovered port on the leukopak and ensure the spike is correctly inserted*.
*alternatively, if the leukopak has an existing transfer line with a spike, this can be used to transfer the material to Bag A via an unused port.

5. Mobilise the contents of the leukopak by gently massaging for 10s and then drain the material into Bag A via the spike connection and seal the transfer tube with a clip.

6. Optionally seal the tubing connecting the two bags and detach them from each other.



7. Mix the contents of Bag A by gently massaging the material for 30s, whilst inverting the bag to ensure the contents are fully mixed.

Stage 2

8. Ensure that the beads in Bag B are well separated by gently massaging the beads.

9. Remove the protective tab from an unused port on Bag A.

10. Remove the protective cover from the spike attached to Bag B. Spike the uncovered port on Bag A and ensure the spike is correctly inserted.

11. Mobilise the contents of Bag A by gently massaging for 10s, and then remove the clip from the line connecting Bag A to Bag B. Drain the material into Bag B via the spike connection, and then replace the clip at Bag B.

12. Seal the tubing connecting the two bags and then detach them from each other.

13. Mix the contents of Bag B by placing on a rocker set to 30rpm for 15 minutes.

14. Lay Bag B flat on a bench and leave for 1 hour at room temperature, avoiding agitation.

15. Bag B alone needs to be shipped; the other materials can be discarded in the appropriate manner.

Stage 3

16. Prepare Bag B for shipping. Place Bag B inside a category B biological substance bag with some absorbent pads.

17. Seal the bag and then lay it flat inside a shipping container along with Bag C (Dissolution Buffer).

18. Store away from light in a polystyrene box at the recommended temperature for the cell type encapsulation (see section 6.2). Alternatively, for the most up to date recommendations on storage temperatures and times per immune cell subpopulation, visit our Compatibility section on our website or contact sales@atelerix.co.uk.

[1]Use the LeukoStor Bag containing beads provided for encapsulation, storage, and release.

6.2. CELL DENSITY AND LOADING GUIDE

The below recommendations yield the highest viable recovery of the indicated cell type. To preserve all leukocyte populations, we recommend storing at 25°C.

Cell Type	Recommended Storage Conditions	Testing Time of Encapsulation
T-Cells	25°C	5 Days
NK-Cells	25°C	5 Days
B-Cells	2-8°C	5 Days
Monocytes	2-8°C	5 Days

If you cannot find any recommendations for your cell type, please contact Sales@atelerix.co.uk.

6.3. RELEASE

Stage 1

1. Remove Bag B and Bag C (Dissolution Buffer) from the shipping container.
2. Remove the protective tab from an unused port on Bag B.
3. Remove the protective cover from the spike attached to Bag C. Spike the unused port on Bag B and ensure the spike is correctly inserted.
4. Remove the clip on Bag C before draining the dissolution buffer from Bag C into Bag B via the spike connection and then replace the clip close to Bag B.

Stage 2

5. Remove Bag B from the rocker and place in a Class II Microbiological Safety Cabinet.
6. Take the Blood Administration Set from its packaging and ensure the tube is clamped, then remove the protective cover from its spike.
7. Remove existing spike from the port in Bag B and insert the spike from the Blood Administration Set.

8. Hang Bag B at a height.

9. Remove cap from the Blood Administration Set line.

10. Unclamp the line and allow to strain into collection vessels ensuring enough space remains in the vessels for the addition of an equal volume of wash buffer (i.e. if draining into 50 mL centrifuge tubes, add 25 mL dissolved leukopak material).

11. Add an equal volume of wash buffer to the dissolved leukopak material (i.e. if draining into 50 mL centrifuge tubes, add 25 mL wash buffer).

12. Washed leukopak material can now be used directly for downstream processing or sedimented to retrieve leukocytes*. ***Sedimentation using a centrifuge set to 350 x g for 10 minutes with soft deceleration is recommended.**

6.4. SHIPPING YOUR SAMPLE

Use appropriate controlled temperature packaging[2] when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

[2]For best cell recovery upon arrival, we recommend using the ICECATCH Solid Ambient or Cool Shipping Boxes.

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7. TROUBLESHOOTING GUIDE

Problem / Question	Guidance
I have air bubbles in the gel after mixing with my sample is this a problem?	Air trapped within the gel will affect preservation, so bubbles should be eliminated before mixing with the beads. Allow time for the mixture to settle and the bubbles to travel to the surface before addition.
Can I ship the Dissolution Buffer in the same package as the samples?	Yes, the Dissolution Buffer is stable at a wide range of temperatures and can be shipped together with the encapsulated samples.
What are the recommended storage times and temperatures for my cell type?	A guide to the recommended storage times and temperatures can be found on our Compatibility section on our website. If you cannot find any recommendations for your cell type, please contact Sales@atelerix.co.uk.
Can I reuse the contents of the kit if I don't use it all?	No, there should only be sufficient volume for a set number of encapsulations per kit. Any spare reagents will not be sufficient to perform any additional encapsulations properly.
Can I split the kit into smaller tubes to get more encapsulations?	No, we do not recommend removing the beads from the tubes supplied, or deviating from the volumes stated.

8. STATEMENTS

8.1. KIT STORAGE AND STABILITY

This kit is stable at 2-8°C for up to 6 months. Atelerix does not recommend using the kit after the expiry date stated on the packaging.

8.2. CELLULAR MATERIAL

Please ensure that apheresis material is free of fungal and bacterial contamination before proceeding.

8.3. TRADEMARKS

LeukoStor™ is a trademark of Atelerix Ltd.

NOTES