



LS-10; LS-30; LS-70

Preservation of Leukapheresis

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1 Components

1.1 Kit Contents

Product Code	Components	Units	Unit Volume	Apheresis Volume to add
LS-10S-03	Gel A (5x)	3 tubes	2.5 mL	10.0 mL
	GelBase Beads	3 tubes	10.0 mL	-
	Dissolution Buffer	3 tubes	20.0 mL	-
	Cell Strainer	6 units	-	-
LS-10S-06	Gel A (5x)	6 tubes	2.5 mL	10.0 mL
	GelBase Beads	6 tubes	10.0 mL	-
	Dissolution Buffer	6 tubes	20.0 mL	-
	Cell Strainer	12 units	-	-
LS-10S-12	Gel A (5x)	12 tubes	2.5 mL	10.0 mL
	GelBase Beads	12 tubes	10.0 mL	-
	Dissolution Buffer	12 tubes	20.0 mL	-
	Cell Strainer	24 units	-	-
LS-10S-24	Gel A (5x)	24 tubes	2.5 mL	10.0 mL
	GelBase Beads	24 tubes	10.0 mL	-
	Dissolution Buffer	24 tubes	20.0 mL	-
	Cell Strainer	48 units	-	-
LS-10S-50	Gel A (5x)	50 tubes	2.5 mL	10.0 mL
	GelBase Beads	50 tubes	10.0 mL	-
	Dissolution Buffer	50 tubes	20.0 mL	-
	Cell Strainer	100 units	-	-
LS-30S-03	Bag A (5x Gel A)	3 bags	7.5 mL	30.0 mL
	Bag B (GelBASE beads)	3 bags	30.0 mL	-
	Bag C (Dissolution Buffer)	3 bags	45.0 mL	-
	Blood administration set	3 units	-	-
	Line clamps	9 units	-	-
LS-30S-06	Bag A (5x Gel A)	6 bags	7.5 mL	30.0 mL
	Bag B (GelBASE beads)	6 bags	30.0 mL	-
	Bag C (Dissolution Buffer)	6 bags	45.0 mL	-
	Blood administration set	6 units	-	-
	Line clamps	18 units	-	-

Product Code	Components	Units	Unit Volume	Apheresis Volume to add
LS-30S-12	Bag A (5x Gel A)	12 bags	7.5 mL	30.0 mL
	Bag B (GelBASE beads)	12 bags	30.0 mL	-
	Bag C (Dissolution Buffer)	12 bags	45.0 mL	-
	Blood administration set	12 units	-	-
	Line clamps	36 units	-	-
LS-30S-24	Bag A (5x Gel A)	24 bags	7.5 mL	30.0 mL
	Bag B (GelBASE beads)	24 bags	30.0 mL	-
	Bag C (Dissolution Buffer)	24 bags	45.0 mL	-
	Blood administration set	24 units	-	-
	Line clamps	72 units	-	-
LS-30S-50	Bag A (5x Gel A)	50 bags	7.5 mL	30.0 mL
	Bag B (GelBASE beads)	50 bags	30.0 mL	-
	Bag C (Dissolution Buffer)	50 bags	45.0 mL	-
	Blood administration set	50 units	-	-
	Line clamps	150 units	-	-
LS-70S-03	Bag A (5x Gel A)	3 bags	17.5 mL	70.0 mL
	Bag B (GelBASE beads)	3 bags	70.0 mL	-
	Bag C (Dissolution Buffer)	3 bags	105.0 mL	-
	Blood administration set	3 units	-	-
	Line clamps	9 units	-	-
LS-70S-06	Bag A (5x Gel A)	6 bags	17.5 mL	70.0 mL
	Bag B (GelBASE beads)	6 bags	70.0 mL	-
	Bag C (Dissolution Buffer)	6 bags	105.0 mL	-
	Blood administration set	6 units	-	-
	Line clamps	18 units	-	-
LS-70S-12	Bag A (5x Gel A)	12 bags	17.5 mL	70.0 mL
	Bag B (GelBASE beads)	12 bags	70.0 mL	-
	Bag C (Dissolution Buffer)	12 bags	105.0 mL	-
	Blood administration set	12 units	-	-
	Line clamps	36 units	-	-
LS-70S-24	Bag A (5x Gel A)	24 bags	17.5 mL	70.0 mL
	Bag B (GelBASE beads)	24 bags	70.0 mL	-
	Bag C (Dissolution Buffer)	24 bags	105.0 mL	-
	Blood administration set	24 units	-	-
	Line clamps	72 units	-	-

Product Code	Components	Units	Unit Volume	Apheresis Volume to add
LS-70S-50	Bag A (5x Gel A)	50 bags	17.5 mL	70.0 mL
	Bag B (GelBASE beads)	50 bags	70.0 mL	-
	Bag C (Dissolution Buffer)	50 bags	105.0 mL	-
	Blood administration set	50 units	-	-
	Line clamps	150 units	-	-

NOTE: Remove components from 2-8°C storage 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*)

Laboratory rocker

Class II microbiological safety cabinet (*optional*)

500 mL polystyrene storage containers (*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*

4.3, 5.3 or 6.3)

2 Before You Begin using LeukoStor™

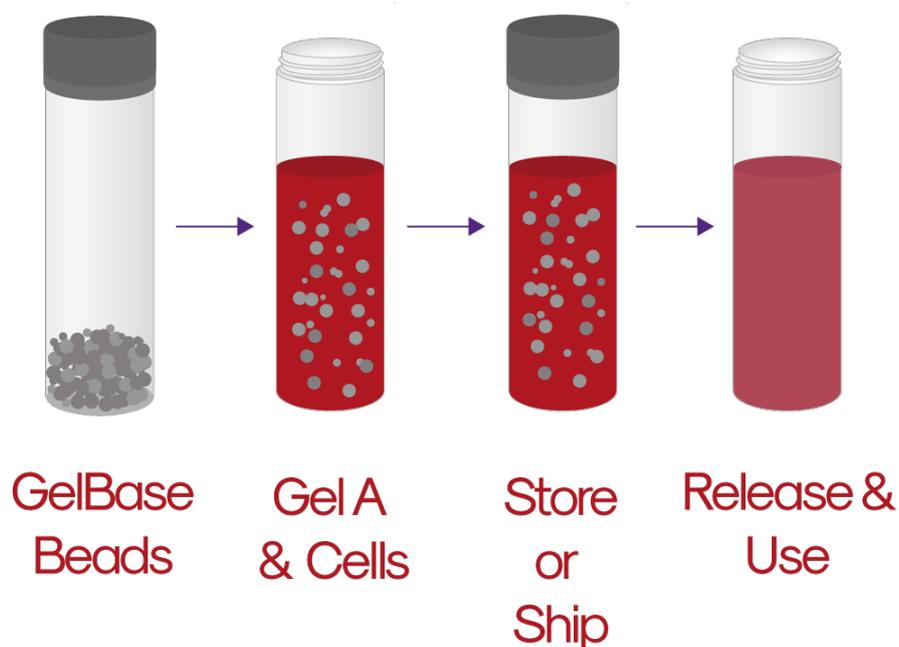
1. If you have not already, we recommend you fill in our Technical Support Questionnaire to get tailored support for your cell type. Fill in the questionnaire at <https://www.atelerix.co.uk/technical-support-questionnaire/>.
2. Ensure LeukoStor™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.
3. Read the troubleshooting guide on page 17 to see our list of frequently asked questions. For any further queries, please email us at technical@atelerix.co.uk.
4. LeukoStor™ is intended for use solely in accordance with this protocol using the components provided within the kit.

3 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	1-2 x 10 ⁹ cells

4 LeukoStor™-10 Step-by-Step Guide

4.1 Overview



4.2 Gelation

Stage 1

1. Remove **Gel A** from refrigerated storage and allow to equilibrate to room temperature for at least 30 minutes.
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

1. Transfer 12.5 mL of **Gel A** / leukapheresis mixture into the tube containing **GelBASE beads** using a 10 mL or 25 mL serological pipette.

2. Agitate the tube by hand to ensure full distribution of beads throughout the leukapheresis material.

Stage 3

1. Prepare sample for shipping. Place inside a category B biological substance bag with some absorbent pads.
2. Seal the bag and then lay it flat inside the outer shipping container.
3. Ship and/or store the apheresis material at warm room temperature (20 – 25 °C), unless otherwise specified (*See Section 4.3 for a storage temperature guide*).
4. Store away from light at the recommended temperature for the cell type encapsulated. See the table below or, for the most up to date recommendations on storage temperatures and times, please check our dedicated webpage by visiting <https://www.atelerix.co.uk/guidelines-for-testing-conditions/> or by scanning the QR code.



4.3 Storage Temperature 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 technical@atelerix.co.uk.

4.4 Release

Stage 1

1. Remove **Dissolution Buffer** from 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 apheresis 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 apheresis 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

1. Place tube containing dissolved apheresis material in a Class II Microbiological Safety Cabinet.
2. Prepare 2 x 50 mL centrifuge tubes placing cell strainers onto the uncapped tubes.
3. 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 material to flow through the strainer.
4. Once all apheresis has been strained, wash **GelBASE beads** in the strainer with 10 mL leukopak wash buffer using a 10 mL serological pipette.
5. Dispose of cell strainers containing **GelBASE beads** via the appropriate route.
6. Sediment washed apheresis material using a centrifuge set to 350 x g for 10 minutes with soft deceleration.
7. Resuspend leukapheresis material with a suitable volume of cell culture medium ready for downstream processes.

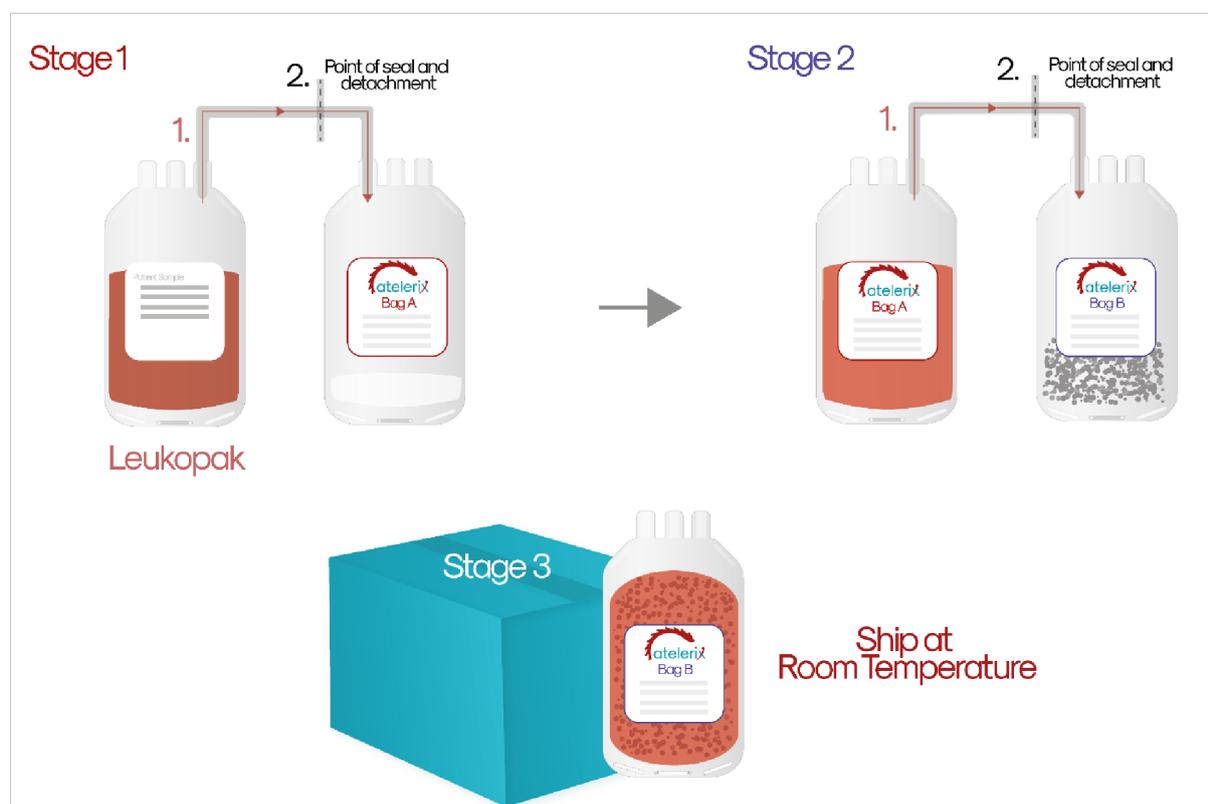
4.5 Shipping Your Samples

Use appropriate controlled room temperature packaging² when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

²For best cell recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at <http://pelicanbiothermal.com/thermal-packaging>.

5 LeukoStor™-30 Step-by-Step Guide

5.1 Overview



5.2 Gelation

Stage 1

1. 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.
2. Remove the protective tab from an unused port on the leukopak.
3. 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 or material can be transferred into an unused port using a serological pipette with pipette controller.

4. 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.
5. Optionally seal the tubing connecting the two bags and detach them from each other.
6. 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

1. Ensure beads in **Bag B** are well separated by gently massaging the beads.
2. Remove the protective tab from an unused port on **Bag A**.
3. 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.
4. 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**.
5. Seal the tubing connecting the two bags and then detach them from each other.
6. Mix the contents of **Bag B** by placing on a rocker set to 30rpm for 15min.
7. Lay **Bag B** flat on a bench and leave for 1h at room temperature, avoiding agitation.
8. **Bag B** alone needs to be shipped; the other materials can be discarded in the appropriate manner.

Stage 3

1. Prepare **Bag B** for shipping. Place **Bag B** inside a category B biological substance bag with some absorbent pads.
2. Seal the bag and then lay it flat inside the outer shipping container along with **Bag C** (Dissolution Buffer).
3. Ship and/or store the leukopak at warm room temperature (20 – 25 °C), unless otherwise specified (*See Section 5.3 for a storage temperature guide*).

4. Store away from light at the recommended temperature for the cell type encapsulated. See the table below or, for the most up to date recommendations on storage temperatures and times, please check our dedicated webpage by visiting <https://www.atelerix.co.uk/guidelines-for-testingconditions/> or by scanning the QR code.



5.3 Storage Temperature Guide

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 technical@atelerix.co.uk.

5.4 Release

Stage 1

1. Remove Bag B (gelled apheresis material) 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.

5. Optionally seal the tubing connecting the two bags and then detach them from each other.
6. Mix the contents of Bag B by placing on a rocker set to 40rpm for 45 minutes to dissolve the gel encapsulating leukapheresis. If any gel remains, gently dissociate the gel by massaging.

Stage 2

1. Remove Bag B from the rocker and place in a Class II Microbiological Safety Cabinet.
2. Take the blood administration set from its packing and ensure the tube is clamped, remove the protective cover from its spike.
3. Remove existing spike from the port in Bag B and insert the spike from the blood administration kit.
4. Hang Bag B at a height.
5. Remove cap from the blood administration set line.
6. 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).
7. 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).
8. 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.

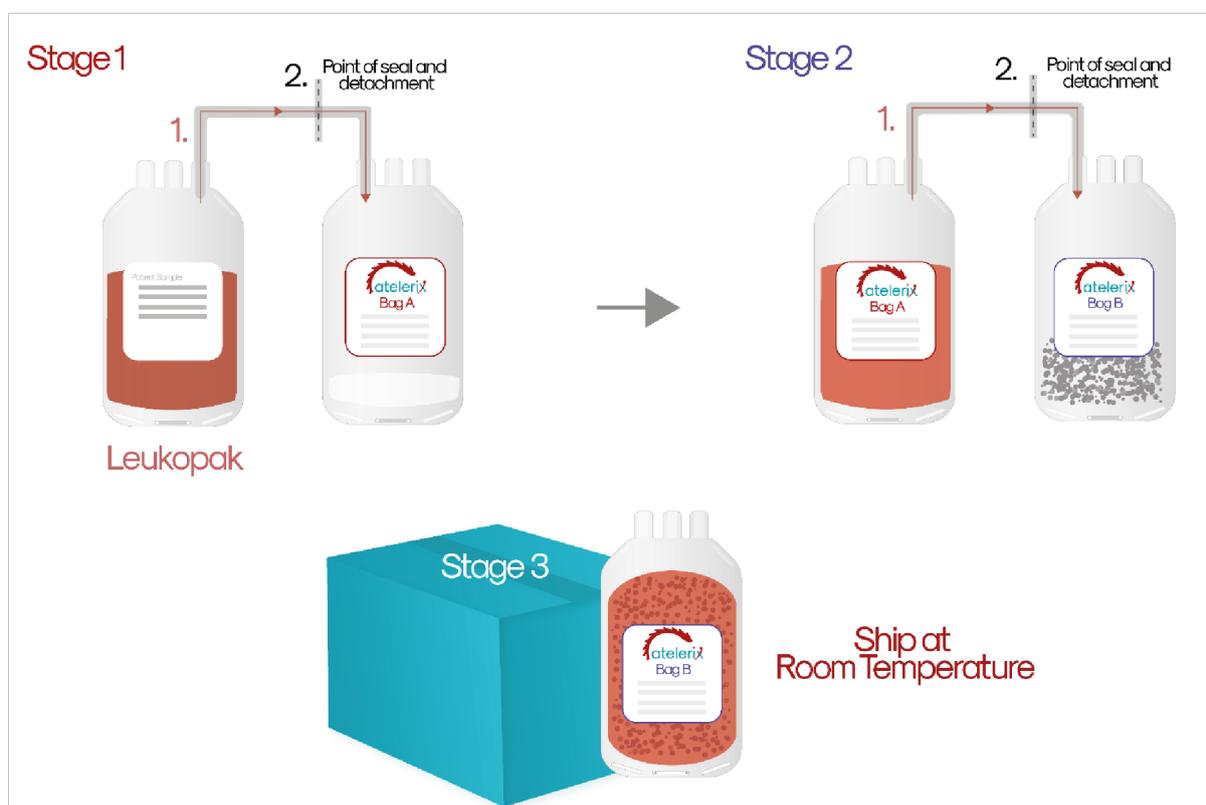
5.5 Shipping Your Samples

Use appropriate controlled room temperature packaging² when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

²For best cell recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at <http://pelicanbiothermal.com/thermal-packaging>.

6 LeukoStor™-70 Step-by-Step Guide

6.1 Overview



6.2 Gelation

Stage 1

1. 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.
2. Remove the protective tab from an unused port on the leukopak.
3. 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.

4. 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.
5. Optionally seal the tubing connecting the two bags and detach them from each other.
6. 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

1. Ensure beads in **Bag B** are well separated by gently massaging the beads.
2. Remove the protective tab from an unused port on **Bag A**.
3. 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.
4. 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**.
5. Seal the tubing connecting the two bags and then detach them from each other.
6. Mix the contents of **Bag B** by placing on a rocker set to 30rpm for 15min.
7. Lay **Bag B** flat on a bench and leave for 1h at room temperature, avoiding agitation.
8. **Bag B** alone needs to be shipped; the other materials can be discarded in the appropriate manner.

Stage 3

1. Prepare **Bag B** for shipping. Place **Bag B** inside a category B biological substance bag with some absorbent pads.
2. Seal the bag and then lay it flat inside a shipping container along with **Bag C** (Dissolution Buffer).
3. Ship and/or store the leukopak at warm room temperature (20 – 25 °C), unless otherwise specified (*See Section 6.3 for a storage temperature guide*).

4. Store away from light at the recommended temperature for the cell type encapsulated. See the table overleaf or, for the most up to date recommendations on storage temperatures and times, please check our dedicated webpage by visiting <https://www.atelerix.co.uk/guidelines-for-testingconditions/> or by scanning the QR code.



6.3 Storage Temperature Guide

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 technical@atelerix.co.uk.

6.4 Release

Stage 1

1. Remove **Bag B** (gelled apheresis material) 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.
5. Optionally seal the tubing connecting the two bags and then detach them from each other.
6. Mix the contents of Bag B by placing on a rocker set to 40rpm for 45 minutes to dissolve the gel encapsulating leukapheresis. If any gel remains, gently dissociate the gel by massaging.

Stage 2

1. Remove Bag B from the rocker and place in a Class II Microbiological Safety Cabinet.
2. Take the blood administration set from its packing and ensure the tube is clamped, remove the protective cover from its spike.
3. Remove existing spike from the port in Bag B and insert the spike from the blood administration kit.
4. Hang Bag B at a height.
5. Remove cap from the blood administration set line.
6. 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).
7. 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).
8. 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.

6.5 Shipping Your Samples

Use appropriate controlled room temperature packaging² when preparing cells for shipping to reduce the effect of ambient temperature change on the encapsulated cells during transit.

²For best cell recovery upon arrival, we recommend using the CoolGuard™ Advance CRT container. Find out more at <http://pelicanbiothermal.com/thermal-packaging>.

7 Troubleshooting Guide

Problem / Question	Guidance
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 in sections 4.3, 5.3 or 6.3 of this book and at https://www.atelerix.co.uk/guidelines-for-testing-conditions/ . If you cannot find any recommendations for your cell type please contact technical@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 use PBS instead of media when encapsulating samples?	No, PBS should not be used at any point as it inhibits and slowly reverses gelation.
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 6 months. Atelerix does not recommend using the kit after the expiry date stated on the packaging.

8.2 Cellular Material

Please ensure that cell cultures are free of fungal and bacterial contamination before proceeding.

8.3 Trademarks

LeukoStor™ is a trademark of Atelerix Ltd.

Notes