

# **BLOODREADY**<sup>TM</sup>

### BLR-005S; BLR-01S; BLR-03S; BLR-05S; BLR-10S

**PRESERVATION OF WHOLE BLOOD** 

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BloodReady™ Handbook BLR01

## **1** Components

### 1.1 Kit contents

Product Code	Components	Units	Unit Volume	Medium to Add
	GelBase Beads	3 tubes	0.5 mL	-
BLR-005-03	Gel A (5x)	3 tubes	0.125 mL	0.5 mL
BLR-003-03	<b>Dissolution Buffer</b>	3 tubes	1 mL	-
	MACS SmartStrainers	3		
	GelBase Beads	6 tube	0.5 mL	-
BLR-005-06	Gel A (5x)	6 tube	0.125 mL	0.5 mL
BLR-005-06	Dissolution Buffer	6 tube	1 mL	-
	MACS SmartStrainers	6		
	GelBase Beads	12 tube	0.5 mL	-
BLR-005-12	Gel A (5x)	12 tube	0.125 mL	0.5 mL
DLK-005-12	Dissolution Buffer	12 tube	1 mL	-
	MACS SmartStrainers	12		
	GelBase Beads	24 tube	0.5 mL	-
	Gel A (5x)	24 tube	0.125 mL	0.5 mL
BLR-005-24	Dissolution Buffer	24 tube	1 mL	-
	MACS SmartStrainers	24		
	GelBase Beads	50 tube	0.5 mL	-
	Gel A (5x)	50 tube	0.125 mL	0.5 mL
BLR-005-50	Dissolution Buffer	50 tube	1 mL	-
	MACS SmartStrainers	50		
	GelBase Beads	3 tube	1 mL	-
	Gel A (5x)	3 tube	0.25 mL	1 mL
BLR-01S-03	<b>Dissolution Buffer</b>	3 tube	2 mL	-
	MACS SmartStrainers	3		
	GelBase Beads	6 tube	1 mL	-
	Gel A (5x)	6 tube	0.25 mL	1 mL
BLR-01S-06	Dissolution Buffer	6 tube	2 mL	-
	MACS SmartStrainers	6		

Product Code	Components	Units	Unit Volume	Medium to Add
	GelBase Beads	12 tube	1 mL	-
BLR-01S-12	Gel A (5x)	12 tube	0.25 mL	1.0 mL
BLN-013-12	<b>Dissolution Buffer</b>	12 tube	2 mL	-
	MACS SmartStrainers	12		
	GelBase Beads	24 tube	1 mL	-
BLR-01S-24	Gel A (5x)	24 tube	0.25 mL	1.0 mL
BLR-013-24	Dissolution Buffer	24 tube	2 mL	-
	MACS SmartStrainers	24		
	GelBase Beads	50 tube	1 mL	-
BLR-01S-50	Gel A (5x)	50 tube	0.25 mL	1.0 mL
BLN-013-50	<b>Dissolution Buffer</b>	50 tube	2 mL	-
	MACS SmartStrainers	50		
	GelBase Beads	3 tube	3 mL	-
BLR-03S-03	Gel A (5x)	3 tube	0.75 mL	3.0 mL
DLR-035-03	Dissolution Buffer	3 tube	6 mL	-
	MACS SmartStrainers	3		
	GelBase Beads	6 tube	3 mL	-
BLR-03S-06	Gel A (5x)	6 tube	0.75 mL	3.0 mL
BLN-033-00	Dissolution Buffer	6 tube	6 mL	-
	MACS SmartStrainers	6		
	GelBase Beads	12 tube	3 mL	-
BLR-03S-12	Gel A (5x)	12 tube	0.75 mL	3.0 mL
DLR-035-12	<b>Dissolution Buffer</b>	12 tube	6 mL	-
	MACS SmartStrainers	12		
	GelBase Beads	24 tube	3 mL	-
	Gel A (5x)	24 tube	0.75 mL	3.0 mL
BLR-03S-24	Dissolution Buffer	24 tube	6 mL	-
	MACS SmartStrainers	24		
	GelBase Beads	50 tube	3 mL	-
	Gel A (5x)	50 tube	0.75 mL	3.0 mL
BLR-03S-50	Dissolution Buffer	50 tube	6 mL	-
	MACS SmartStrainers	50		

Product Code	ct Code Components		Unit Volume	Medium to Add
	GelBase Beads	3 tube	5 mL	-
BLR-05S-03	Gel A (5x)	3 tube	1.25 mL	5.0 mL
BLN-033-03	<b>Dissolution Buffer</b>	3 tube	10 mL	-
	MACS SmartStrainers	6		
	GelBase Beads	6 tube	5 mL	-
BLR-05S-06	Gel A (5x)	6 tube	1.25 mL	5.0 mL
BLN-033-00	Dissolution Buffer	6 tube	10 mL	-
	MACS SmartStrainers	12		
	GelBase Beads	12 tube	5 mL	-
BLR-05S-12	Gel A (5x)	12 tube	1.25 mL	5.0 mL
BLR-033-12	Dissolution Buffer	12 tube	10 mL	-
	MACS SmartStrainers	24		
	GelBase Beads	24 tube	5 mL	-
	Gel A (5x)	24 tube	1.25 mL	5.0 mL
BLR-05S-24	Dissolution Buffer	24 tube	10 mL	-
	MACS SmartStrainers	48		
	GelBase Beads	50 tube	5 mL	-
	Gel A (5x)	50 tube	1.25 mL	5.0 mL
BLR-05S-50	Dissolution Buffer	50 tube	10 mL	-
	MACS SmartStrainers	100		
	GelBase Beads	3 tube	10 mL	-
BLR-10S-03	Gel A (5x)	3 tube	2.5 mL	10 mL
BER-103-03	Dissolution Buffer	3 tube	20 mL	-
	MACS SmartStrainers	9		
	GelBase Beads	3 tube	10 mL	-
BLR-10S-06	Gel A (5x)	3 tube	2.5 mL	10 mL
BER-103-00	Dissolution Buffer	3 tube	20 mL	-
	MACS SmartStrainers	18		
	GelBase Beads	12 tube	10 mL	-
PL D 100 10	Gel A (5x)	12 tube	2.5 mL	10 mL
BLR-10S-12	<b>Dissolution Buffer</b>	12 tube	20 mL	-
	MACS SmartStrainers	36		
BLR-10S-24	GelBase Beads	24 tube	10 mL	-
BLN-100-24	Gel A (5x)	24 tube	2.5 mL	10 mL
	Dissolution Buffer	24 tube	20 mL	-

Product Code	Components	Units	Unit Volume	Medium to Add
	MACS SmartStrainers	72		
	GelBase Beads	50 tube	10 mL	-
BLR-10S-50	Gel A (5x)	50 tube	2.5 mL	10 mL
DLK-103-50	Dissolution Buffer	50 tube	20 mL	-
	MACS SmartStrainers	150		

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

#### 1.2 Components to be supplied by the user:

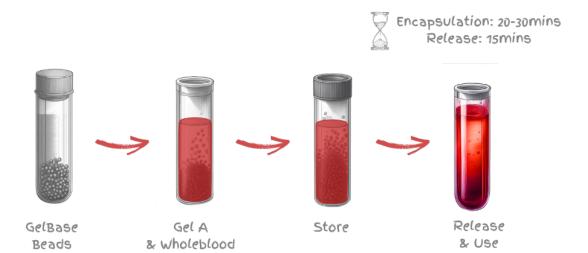
1000 µL pipettes and tips
10 mL, 25 mL serological pipette and pipette aid
Whole Blood (0.5, 1, 3, 5 or 10 mL) with Sodium Heparin
15 mL, 50 mL centrifuge tubes
Cell culture medium or wash buffer
SepMate<sup>™</sup> PBMC isolation tubes

### 2 Before You Begin

- Ensure BloodReady<sup>™</sup> kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.
- Read the troubleshooting guide on page 13 to see our list of frequently asked questions.
   For any further queries, please email us at <u>orders@atelerix.co.uk</u>.
- 3. BloodReady<sup>™</sup> is intended for use solely in accordance with this protocol using the components provided within the kit.

### **3** Protocol Overview

#### 3.1 Overview



## 4 BloodReady<sup>™</sup> (BLR-005S)

#### 4.1 Gelation

- 1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
- 2. Add 0.5 mL of the whole blood sample to the vial containing 0.125 mL Gel A.
- 3. Gently mix until homogenous with a pipette, ensuring that no bubbles are introduced.
- Add 0.625 mL of the blood / Gel A mix slowly to the GelBASE Beads<sup>1</sup> using a 1000 μL 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 the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
- 6. Store away from light in a polystyrene box between 2-8°C.

<sup>1</sup>Use the BloodReady Vial containing **GelBASE Beads** provided for encapsulation, storage, and release.

- 1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
- 2. Remove lids from tubes and use a 1000 µL pipette to infuse 1 mL **Dissolution Buffer** into the bottom of the gel by piercing the gel.
- 3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes.**
- 4. Filter the **GelBASE Beads** out of the released whole blood by pipetting the sample through a MACS<sup>®</sup> SmartStrainer (100 μm) into a 15 mL centrifuge tube.
- 5. To proceed with PBMC isolation, please refer to Page 12.

### 5 BloodReady<sup>™</sup> (BLR-01S)

#### 5.1 Gelation

- 1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
- 2. Add 1 mL of the whole blood sample to the vial containing 0.25 mL Gel A.
- 3. Gently mix until homogenous with a pipette, ensuring that no bubbles are introduced.
- 4. Add 1.25 mL of the blood / Gel A mix slowly to the GelBASE Beads<sup>1</sup> using a 1000 μL 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 the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
- 6. Store away from light in a polystyrene box between 2-8°C.

<sup>1</sup>Use the BloodReady Vial containing **GelBASE Beads** provided for encapsulation, storage, and release.

- 1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
- 2. Remove lids from tubes and use a 1000  $\mu$ L pipette to infuse 2 mL **Dissolution Buffer** into the bottom of the gel by piercing the gel.
- 3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes.**

- 4. Filter the **GelBASE Beads** out of the released whole blood by pipetting the sample through a MACS<sup>®</sup> SmartStrainer (100 μm) into a 15 mL centrifuge tube.
- 5. To proceed with PBMC isolation, please refer to Page 12.

### 6 BloodReady<sup>™</sup> (BLR-03S)

#### 6.1 Gelation

- 1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
- 2. Add 3 mL of the whole blood sample to the vial containing 0.75 mL Gel A.
- 3. Gently mix until homogenous with a pipette, ensuring that no bubbles are introduced.
- Add 3.75 mL of the blood / Gel A mix slowly to the GelBASE Beads<sup>1</sup> using a 1000 μL 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 the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
- 6. Store away from light in a polystyrene box between 2-8°C.

<sup>1</sup>Use the BloodReady Vial containing GelBASE Beads provided for encapsulation, storage, and release.

- 1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
- 2. Remove lids from tubes and use a Pasteur pipette to infuse 6 mL **Dissolution Buffer** into the bottom of the gel by piercing the gel.
- 3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes.**
- 4. Filter the **GelBASE Beads** out of the released whole blood by pipetting the sample through a MACS<sup>®</sup> SmartStrainer (100 μm) into a 50 mL centrifuge tube.

5. To proceed with PBMC isolation, please refer to Page 12.

### 7 BloodReady<sup>™</sup> (BLR-05S)

#### 7.1 Gelation

- 1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
- 2. Add 5 mL of the whole blood sample to the vial containing 1.25 mL Gel A.
- 3. Gently mix until homogenous with a pipette, ensuring that no bubbles are introduced.
- 4. Add 6.25 mL of the blood / Gel A mix slowly to the GelBASE Beads<sup>1</sup> using a Pasteur 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 the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
- 6. Store away from light in a polystyrene box between 2-8°C.

<sup>1</sup>Use the BloodReady Vial containing **GelBASE Beads** provided for encapsulation, storage, and release.

- 1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
- 2. Remove lids from tubes and use a Pasteur pipette to infuse 10 mL **Dissolution Buffer** into the bottom of the gel by piercing the gel.
- 3. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes.**
- 4. Filter the **GelBASE Beads** out of the released whole blood by pipetting the sample through a MACS<sup>®</sup> SmartStrainer (100 μm) into a 50 mL centrifuge tube.
- 5. To proceed with PBMC isolation, please refer to Page 12.

### 8 BloodReady<sup>™</sup> (BLR-10S)

#### 8.1 Gelation

- 1. Ensure that all components are allowed to equilibrate to room temperature before use and that gels are at the bottom of their tubes. Conduct all steps in a laminar flow hood at room temperature.
- 2. Add 10 mL of the whole blood sample to the vial containing 2.5 mL Gel A.
- 3. Gently mix until homogenous with a pipette, ensuring that no bubbles are introduced.
- 4. Add 12.5 mL of the blood / Gel A mix slowly to the GelBASE Beads<sup>1</sup> using a 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 the contents, ensuring a tight seal (the gel will cure *in situ* within approximately 30 minutes, sample is ready to ship after 1 hour).
- 6. Store away from light in a polystyrene box between 2-8°C.

<sup>1</sup>Use the BloodReady Vial containing **GelBASE Beads** provided for encapsulation, storage, and release.

- 1. Ensure that all components and samples are allowed to equilibrate to room temperature before use and conduct all steps in a laminar flow hood at room temperature.
- 2. Transfer the encapsulated whole blood sample into a 50 mL falcon tube by removing the lid from the tube and squeezing the tube to dislodge the gelled sample. This will allow a sufficient volume capacity for the subsequent addition of Dissolution Buffer.
- 3. Remove lids from tubes and use a Pasteur pipette to infuse 20 mL **Dissolution Buffer** into the bottom of the gel by piercing the gel.
- 4. Place the cap back on the tube and allow the gel to dissolve by occasionally agitating the tube by gentle inversion or rocking for **10 minutes.**
- 5. Filter the **GelBASE Beads** out of the released whole blood by pipetting the sample through a MACS<sup>®</sup> SmartStrainer (100 μm) into a 50 mL centrifuge tube.
- 6. To proceed with PBMC isolation, please refer to Page 12.

### 9 **PBMC** Isolation

#### 9.1 PBMC Isolation using SepMate<sup>™</sup> Tubes

For peripheral blood mononuclear cell (PBMC) isolation following whole blood release, Atelerix recommends the use of SepMate<sup>™</sup> PBMC Isolation Tubes, which enable consistent and hasslefree PBMC isolation in just 15 minutes. The Product Information Sheet and Directions for Use can be found at: <u>https://www.stemcell.com/products/sepmate-50-ivd.html</u>

The recommended SepMate tube size and Density Gradient Medium volumes for each respective BloodReady volume are stated below:

BloodReady Product	SepMate Tube	Initial Blood Sample (mL)	Final Sample Volume after dilution with equal	Density Gradient
			volume of PBS + 2% FBS.	Medium (mL)
BloodReady™	15	0.5	4.25	4.5
(BLR-005S)				
BloodReady™	15	1.0	8.5	3.5
(BLR-01S)				
BloodReady™	50	3.0	25.4	15
(BLR-03S)				
BloodReady™	50	5.0	42.5	15
(BLR-05S)			*split volume into 2x	
			SepMate tubes	
BloodReady™	50	10.0	85	15
(BLR-10S)			*split volume into 3x	
			SepMate tubes	

 Table 1. Sample and Density Gradient Medium Volumes for PBMC isolation.

#### 9.2 PBMC Viability Assessment Recommendations

For assessing cell viability using Flow Cytometry after whole blood storage in BloodReady, Atelerix recommends the use of 7-AAD over PI for optimum accuracy.

## **10 Troubleshooting Guide**

Problem / Question	Guidance
I have air bubbles in the gel after mixing with my blood 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 blood sample?	Atelerix recommends the storage of whole blood for 3 days at 2-8°C.
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 Gelbase Beads from the tubes supplied or deviating from the volumes stated.
Can I encapsulate higher or lower blood volumes in the same tube in order to get more encapsulations?	Our BloodReady kits have been designed and manufactured to permit encapsulation of the blood volume stated on the kit, please use the volumes stated on pages 1-4.

## **11 Statements**

#### 11.1 Kit storage and stability

This kit is stable at 4°C for 6 months. Bring components up to room temperature before use. Atelerix does not recommend using the kit after the expiry date stated on the packaging.

#### 11.2 Cellular material

Human whole blood specimens can be used. Please ensure that samples are free of fungal and bacteriological contamination before proceeding.

#### 11.3 Trademarks

BloodReady<sup>™</sup> is a trademark of Atelerix Ltd.