BLOODREADY™

PRESERVATION OF WHOLE BLOOD

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

Atelerix Handbook Series

Version BLR01;0.1.1

TABLE OF CONTENTS

1. COMPONENTS

1.1. KIT CONTENTS

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	BLOOD VOLUME TO ADD
BLR-005-03	GelBase Beads	3 Tubes	0.5 mL	-
	Gel A (5x)	3 Tubes	0.125 mL	0.5 mL
	Dissolution Buffer	3 Tubes	1 mL	-
	MACS SmartStrainers	3	-	-
BLR-005-06	GelBase Beads	6 Tubes	0.5 mL	-
	Gel A (5x)	6 Tubes	0.125 mL	0.5 mL
	Dissolution Buffer	6 Tubes	1 mL	-
	MACS SmartStrainers	6	-	-
BLR-005-12	GelBase Beads	12 Tubes	0.5 mL	-
	Gel A (5x)	12 Tubes	0.125 mL	0.5 mL
	Dissolution Buffer	12 Tubes	1 mL	-
	MACS SmartStrainers	12	-	-
BLR-005-24	GelBase Beads	24 Tubes	0.5 mL	-
	Gel A (5x)	24 Tubes	0.125 mL	0.5 mL
	Dissolution Buffer	24 Tubes	1 mL	-
	MACS SmartStrainers	24	-	-
BLR-005-50	GelBase Beads	50 Tubes	0.5 mL	-
	Gel A (5x)	50 Tubes	0.125 mL	0.5 mL
	Dissolution Buffer	50 Tubes	1 mL	-
	MACS SmartStrainers	50	-	-
BLR-01S-03	GelBase Beads	3 Tubes	1 mL	-
	Gel A (5x)	3 Tubes	0.25 mL	1 mL
	Dissolution Buffer	3 Tubes	2 mL	-
	MACS SmartStrainers	3	-	-
BLR-01S-06	GelBase Beads	6 Tubes	1 mL	-
	Gel A (5x)	6 Tubes	0.25 mL	1 mL
	Dissolution Buffer	6 Tubes	2 mL	-
	MACS SmartStrainers	6	-	-
BLR-01S-12	GelBase Beads	12 Tubes	1 mL	-
	Gel A (5x)	12 Tubes	0.25 mL	1 mL
	Dissolution Buffer	12 Tubes	2 mL	-
	MACS SmartStrainers	12	-	-

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	BLOOD VOLUME TO ADD
BLR-01S-24	GelBase Beads	24 Tubes	1 mL	-
	Gel A (5x)	24 Tubes	0.25 mL	1 mL
	Dissolution Buffer	24 Tubes	2 mL	-
	MACS SmartStrainers	24	-	-
BLR-01S-50	GelBase Beads	50 Tubes	1 mL	-
	Gel A (5x)	50 Tubes	0.25 mL	1 mL
	Dissolution Buffer	50 Tubes	2 mL	-
	MACS SmartStrainers	50	-	-
BLR-03S-03	GelBase Beads	3 Tubes	3 mL	-
	Gel A (5x)	3 Tubes	0.75 mL	3 mL
	Dissolution Buffer	3 Tubes	2 mL	-
	MACS SmartStrainers	3	-	-
BLR-03S-06	GelBase Beads	6 Tubes	3 mL	-
	Gel A (5x)	6 Tubes	0.75 mL	3 mL
	Dissolution Buffer	6 Tubes	2 mL	-
	MACS SmartStrainers	6	-	-
BLR-03S-12	GelBase Beads	12 Tubes	3 mL	-
	Gel A (5x)	12 Tubes	0.75 mL	3 mL
	Dissolution Buffer	12 Tubes	2 mL	-
	MACS SmartStrainers	12	-	-
BLR-03S-24	GelBase Beads	24 Tubes	3 mL	-
	Gel A (5x)	24 Tubes	0.75 mL	3 mL
	Dissolution Buffer	24 Tubes	2 mL	-
	MACS SmartStrainers	24	-	-
BLR-03S-50	GelBase Beads	50 Tubes	3 mL	-
	Gel A (5x)	50 Tubes	0.75 mL	3 mL
	Dissolution Buffer	50 Tubes	2 mL	-
	MACS SmartStrainers	50	-	-
BLR-05S-03	GelBase Beads	3 Tubes	5 mL	-
	Gel A (5x)	3 Tubes	1.25 mL	5 mL
	Dissolution Buffer	3 Tubes	10 mL	-
	MACS SmartStrainers	3	-	-
BLR-05S-06	GelBase Beads	6 Tubes	5 mL	-
	Gel A (5x)	6 Tubes	1.25 mL	5 mL
	Dissolution Buffer	6 Tubes	10 mL	-
	MACS SmartStrainers	6	-	-

PRODUCT CODE	COMPONENTS	UNITS	UNIT VOLUME	BLOOD VOLUME TO ADD
BLR-05S-12	GelBase Beads	12 Tubes	5 mL	-
	Gel A (5x)	12 Tubes	1.25 mL	5 mL
	Dissolution Buffer	12 Tubes	10 mL	-
	MACS SmartStrainers	12	-	-
BLR-05S-24	GelBase Beads	24 Tubes	5 mL	-
	Gel A (5x)	24 Tubes	1.25 mL	5 mL
	Dissolution Buffer	24 Tubes	10 mL	-
	MACS SmartStrainers	24	-	-
BLR-05S-50	GelBase Beads	50 Tubes	5 mL	-
	Gel A (5x)	50 Tubes	1.25 mL	5 mL
	Dissolution Buffer	50 Tubes	10 mL	-
	MACS SmartStrainers	50	-	-
BLR-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	3	-	-
BLR-10S-06	GelBase Beads	6 Tubes	10 mL	-
	Gel A (5x)	6 Tubes	2.5 mL	10 mL
	Dissolution Buffer	6 Tubes	20 mL	-
	MACS SmartStrainers	6	-	-
BLR-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	12	-	-
BLR-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	24	-	-
BLR-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	50	-	-

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

1.2. COMPONENTS TO BE SUPPLIED BY THE USER

• 1000µl Pipettes and Tips • 10ml, 25ml serological pipette and pipette aid • 15ml, 50ml centrifuge tubes • Cell culture medium or wash buffer • SepMate PBMC isolation tubes • Lymphoprep • Whole Blood Sample with Anticoagulant!!*

*kits are compatible with heparin-, EDTA-, and citrate-based anticoagulants including ACD-A

1.3. BEFORE YOU BEGIN USING BLOODREADY™

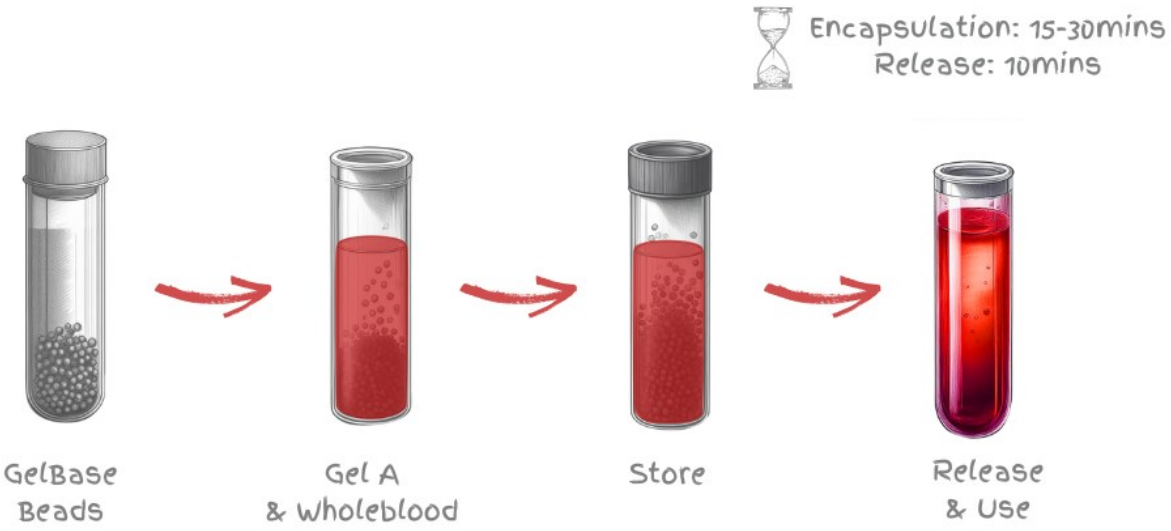
1. Ensure BloodReady™ kits have not passed the expiry date stated on the packaging. Atelerix does not recommend using kits after this date.

2. Read the troubleshooting guide on page 14 to see our list of frequently asked questions. For any further queries, please email us at Sales@atelerix.co.uk.

3. Consult the PBMC isolation protocol on page 13 for retrieval of PBMCs after BloodReady storage of whole blood.

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

2. PROTOCOL OVERVIEW



3. BLOODREADY™ (BLR-005S)

3.1. 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 whole blood to the vial containing 0.125 mL of Gel A.

3. Gently mix until homogenous with a pipette, ensuring that no air bubbles are introduced.

4. Add 0.625 mL of the blood / Gel A solution slowly to the GelBase Beads 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 contents, ensuring a tight seal (the gel will cure *in situ* within approximately 20 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box between 2-8°C. 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 BloodReady Vial containing beads provided for encapsulation, storage, and release.

3.2. 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. Using a serological pipette or syringe with needle, pierce the gel and infuse 1 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 (see troubleshooting guide on page 14).

4. When the gel has fully dissolved, filter the GelBASE Beads out of the released whole blood by pipetting the sample through a MACS® SmartStrainer (100 µm) into a 15 mL centrifuge tube.

5. To proceed with PBMC isolation, please refer to page 13.

3.3. 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

4. BLOODREADY™ (BLR-01S)

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 1 mL of whole blood to the vial containing 0.25 mL of Gel A.

3. Gently mix until homogenous with a pipette, ensuring that no air bubbles are introduced.

4. Add 1.25 mL of the blood / Gel A solution slowly to the GelBase Beads 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 contents, ensuring a tight seal (the gel will cure *in situ* within approximately 20 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box between 2-8°C. 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.

   ¹Use the BloodReady Vial containing beads provided for encapsulation, storage, and release.

4.2. 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. Using a serological pipette or syringe with needle, pierce the gel and infuse 2 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 (see troubleshooting

guide on page 14).

4. When the gel has fully dissolved, Filter the GelBASE Beads out of the released whole blood by pipetting the sample through a MACS® SmartStrainer (100 µm) into a 15 mL centrifuge tube.

5. To proceed with PBMC isolation, please refer to page 13.

4.3. 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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5. BLOODREADY™ (BLR-03S)

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 3 mL of whole blood to the vial containing 0.75 mL of Gel A.

3. Gently mix until homogenous with a pipette, ensuring that no air bubbles are introduced.

4. Add 3.75 mL of the blood / Gel A solution slowly to the GelBase Beads 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 contents, ensuring a tight seal (the gel will cure *in situ* within approximately 20 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box between 2-8°C. 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 BloodReady Vial containing beads provided for encapsulation, storage, and release.

5.2. 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. Using a serological pipette or syringe with needle, pierce the gel and infuse 6 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 (see troubleshooting guide on page 14).

4. When the gel has fully dissolved, filter the GelBASE Beads out of the released whole blood by pipetting the sample through a MACS® SmartStrainer (100 µm) into a 50 mL centrifuge tube.

5. To proceed with PBMC isolation, please refer to page 13.

5.3. 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. BLOODREADY™ (BLR-05S)

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 5 mL of whole blood to the vial containing 1.25 mL of Gel A.

3. Gently mix until homogenous with a pipette, ensuring that no air bubbles are introduced.

4. Add 6.25 mL of the blood / Gel A solution slowly to the GelBase Beads 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 contents, ensuring a tight seal (the gel will cure *in situ* within approximately 20 minutes, sample is ready to ship after 1 hour).

6. Store away from light in a polystyrene box between 2-8°C. 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 BloodReady Vial containing beads provided for encapsulation, storage, and release.

6.2. 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. Using a serological pipette or syringe with needle, pierce the gel and infuse 10 mL Dissolution Buffer into the bottom of the gel by piercing the gel, filling up to the indicated line. As the Dissolution Buffer is added to the gel, remove the pipette tip/needle to avoid spillage and ensure that you do not disturb the sample.

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 (see troubleshooting guide on page 14).

4. When the gel has fully dissolved, filter the GelBASE Beads out of the released whole blood by pipetting the sample through a MACS® SmartStrainer (100 µm) into a 50 mL centrifuge tube.

5. To proceed with PBMC isolation, please refer to page 13.

6.3. 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. BLOODREADY™ (BLR-10S)

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 10 mL of whole blood to the vial containing 2.5 mL of Gel A.

3. Gently mix until homogenous with a pipette, ensuring that no air bubbles are introduced.

4. Add 12.5 mL of the blood / Gel A solution slowly to the GelBase Beads 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 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. 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 BloodReady Vial containing beads provided for encapsulation, storage, and release.

7.2. 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 centrifuge 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 the tubes and use a serological 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 (see troubleshooting guide on page 14).

5. When the gel has fully dissolved, filter the GelBASE Beads out of the released whole blood by pipetting the sample through a MACS® SmartStrainer (100 µm) into a 50 mL centrifuge tube.

6. To proceed with PBMC isolation, please refer to page 13.

7.3. 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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8. PBMC ISOLATION

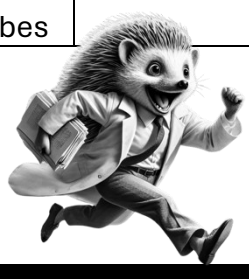
8.1. PBMC ISOLATION USING SEPMATE TUBES

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

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

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





8.2. 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.

9. 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 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 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 1-3.

10. STATEMENTS

10.1. KIT STORAGE AND STABILITY

This kit is stable at 2-8°C for up to 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.

10.2. CELLULAR MATERIAL

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

10.3. TRADEMARKS

BloodReady™ is a trademark of Atelerix Ltd.

NOTES