REF: 9603 - Kit for 16 tests

CellMag™ Epithelial CTC Kit Instructions for Use and Protocol

Original instructions

***NOTICE**: For safe and proper use, read this document, the CellMag Instructions for Use and the CellMag Consumable Instructions for Use. Please keep them for future reference.*

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Contents

INFORMATION ON THIS DOCUMENT

Purpose of the document

This document provides instructions on how to use the CellMag Epithelial CTC Kit together with CellMag Consumable and CellMag to enrich and stain target cells from blood samples.

Related documents

The instructions contained in this protocol are summarized for easy reference in the CellMag Epithelial CTC Kit - Protocol Quick Guide.

PRODUCT DESCRIPTION

Intended use

For Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

Product overview

Cancer metastasis occurs when cells are shed from the primary tumor, enter the circulation and begin to grow in distant locations in the body. Malignant carcinomas are derived from epithelial cells (Cancer Biology, 3rd edition, Ray Ruddon 1995) that are not normally found in circulation. The CellMag Epithelial CTC Kit contains reagents and supplies for immunomagnetic selection of rare circulating epithelial cells from whole blood.

The CellMag Epithelial CTC Kit is intended to be used with CellMag for the immunomagnetic selection and staining of epithelial cells. Target cells can be analyzed on a variety of platforms such as flow cytometry, fluorescent microscopy, PCR and the DEPArray platform.

The CellMag Epithelial CTC Kit contains a ferrofluid-based capture reagent which consists of particles with a magnetic core surrounded by a polymeric layer. The polymeric layer is coated with antibodies targeting the Epithelial Cell Adhesion Molecule (EpCAM) antigen for capturing circulating epithelial cells. After immunomagnetic capture and enrichment, fluorescent staining reagents are added for identification. Anti-CKPE is specific for the intracellular protein cytokeratin (specific for epithelial cells), DAPI stains the cell nucleus, and anti-CD45-APC is specific for leukocytes.

Limitations of the procedures

- For Research Use Only. Not for use in diagnostic procedures. Results should not be used for patient management.
- CTCs that express EpCAM but not cytokeratins 8, 18, and 19 will not be captured by the CellMag Epithelial CTC Kit.
- This is a CellMag assay and is not to be confused with the CellSearch test/system.
- Additional user-defined reagents not included in this kit will need to be optimized at the user's discretion.

Contents

The contents of the package are the following:

- This document
- 16 CELLSEARCH Conical Centrifuge tubes (15 ml)
- 16 Conical tube caps (x 2)
- 9 reagent bottles

Reagent bottle details

Following are the details of the provided bottles:

Name	Color	Volume	Content
Anti-EpCAM Ferrofluid	brown cap	3.0 ml	A suspension of 0.022% magnetic particles conjugated to a mouse monoclonal antibody specific for the cell surface marker EpCAM present on epithelial cells in a buffer containing 0.03% bovine serum albumin (BSA) and 0.05% ProClin 300 preservative
Staining Reagent	white cap	3.0 ml	0.0006% mouse monoclonal antibodies specific to cytokeratins conjugated to phycoerythrin (PE); 0.0012% mouse anti-CD45 monoclonal antibody conjugated to allophycocyanin (APC) in buffer containing 0.5% BSA and 0.1% sodium azide

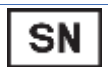
Name	Color	Volume	Content
Nucleic Acid Dye	blue cap	3.0 ml	• 0.005% 4' 6-diamidino-2-phenylindole dihydrochloride (DAPI) • 0.05% ProClin 300
Capture Enhancement Reagent	clear cap	3.0 ml	• 0.02% proprietary reagent for controlled ferrofluid aggregation • 0.5% BSA • 0.1% sodium azide in buffer
Permeabilization Reagent	green cap	3.0 ml	• 0.011% proprietary permeabilization reagent • 0.1% sodium azide in buffer
Cell Fixative	red cap	3.0 ml	• 25% proprietary fixative ingredients • 0.1% BSA • 0.1% sodium azide in buffer
Dilution Buffer	amber bottles and caps	2 bottles × 110 ml	Buffer with 0.1% sodium azide
CellMag Buffer	clear bottle with white cap	120 ml	Buffer with 0.1% sodium azide

Interfering substances

The following studies were performed using the CellSearch CTC Kit.

- SKBR-3 cells spiked into blood samples were exposed to potential interfering substances and compared to untreated controls. Toxic levels (five times therapeutic index) of the following cancer drugs, over-the-counter drugs, and other exogenous substances were tested: cyclophosphamide, Mitomycin C, Procrit, biotin, 5-fluorouracil, methotrexate, tamoxifen citrate, paclitaxel, Arimidex, acetaminophen, acetylsalicylic acid, caffeine, dextromethorphan, Aredia, Human Anti-Mouse Antibody (HAMA) type 1, HAMA type 2, Herceptin, and ibuprofen. No significant differences in SKBR-3 cell numbers were detected, indicating that these substances should not interfere with the CellMag Epithelial CTC Kit.
- Doxorubicin is a known interfering substance for the proper interpretation of images.
- Potential interference from lipemia was studied by adding Intralipid to samples at a concentration of 2.6%, which corresponds to greater than 1000 mg/dl triglyceride.
- Samples were lysed to simulate total hemolysis.
- Bilirubin at 7.4 mg/dl, HAMA 1/HAMA 2 and hematocrit from 18-60% were studied.
- Lipemia, hemolysis, icterus and a broad range of hematocrit values should not interfere with the CellMag Epithelial CTC Kit.

Label symbols

	Use by date
	Batch code
	Serial number
	Part number

	Caution
	Manufacturer data
	Contains sufficient for "n" tests
	Temperature limitation
	Consult the Instructions for Use
	Biological risk
	May cause an allergic skin reaction

PROTOCOL

Materials

Material and equipment required

Name	Volume	Recommended supplier	Code
Pipette	Various	• In general: any supplier • For DEPArray Cartridge loading: Eppendorf	-
Serological pipette	Various	Any supplier	-
Low retention dualfilter pipette tip	Various	• In general: any supplier • For DEPArray Cartridge loading: Eppendorf	-
MicroAmp Reaction tube with cap autoclaved (specific if utilizing VR NxT)	0.2 ml	Thermo Fisher Scientific	N8010612
Protein low retention tube	1.5 ml	Any supplier	-
Vortex with minimum rotation speed of 1600 RPM	-	Any supplier	-
Swing bucket centrifuge	for 15 ml tube and 1.5 ml PCR tube	Any supplier	-
Swing bucket centrifuge for plates (specific if utilizing VR NxT)	for 200 µl tube	Any supplier	-
CellSave Preservative Tube	10 ml	Menarini Silicon Biosystems	7900005
DEPArray Buffer for Fixed Cells	-	Menarini Silicon Biosystems	KI0066
CellMag Epithelial CTC Kit	-	Menarini Silicon Biosystems	9603
CellMag Consumable	-	Menarini Silicon Biosystems	CS0432
CellMag	-	Menarini Silicon Biosystems	CS0431
VR NxT (optional)	-	Menarini Silicon Biosystems	DA0650

Safety

Biological risks

- **Observe national regulations, the biological security level of your laboratory, the Material Safety Data Sheets, and the manufacturer's application notes.**
- **Wear Personal Protective Equipment (PPE) as prescribed by your laboratory safety regulations.**
- **Treat and dispose of waste using proper precautions and in accordance with local, state, and federal regulations.**
- **Never pipette by mouth.**

Chemical risks

- Do not breathe reagent vapors.
- Reagents may cause an allergic skin reaction. Avoid contact with skin. In case of contact, wash immediately with plenty of soap and water and get medical advice if skin irritation or rash occur.
- Some of the reagents contain sodium azide preservative. Keep them away from food and drink. If swallowed, seek medical advice immediately and provide the containers or labels.
- Contaminated work clothing should not be allowed out of the workplace and should be washed before reuse.
- Keep reagents out of reach of children.
- Contact of reagents with acids liberates very toxic gas. Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.
- Dispose of reagents and reagent bottles to an approved waste disposal plant. Some of the reagents contain ProClin 300 as a preservative. ProClin 300 is a mixture of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1). For additional information please refer to Safety Data Sheet on www.cellsearchruo.com

Risk for the samples

- Collect blood into a CellSave Preservative Tube only.
- To avoid affecting sample integrity adversely, do not refrigerating samples prior to processing. Samples must be transported and stored at 15–30 °C (59–86 °F).
- Microbial contamination of reagents can cause erroneous results and should be avoided.

Precautions for using reagents

- Warm all reagents at room temperature between 15–30 °C (59–86 °F) before use.

- Protect reagents from exposure to sunlight and do not freeze them.
- Reagents are supplied ready for use. Store unopened at 2–8 °C (36–46 °F).
- After opening, reagents should be stored for no longer than 30 days. Record the date of each reagent first opening.
- After opening, store the Dilution Buffer and CellMag Buffer at room temperature and the other reagents at 2–8 °C (36–46 °F).
- Do not mix and match reagents from different kits.
- To avoid cross-contamination of reagents, each opened reagent must be recapped with its own colored cap.
- Do not use expired reagents.
- Pipette tips must be single use only.

Additional precaution

It is recommended to work under a biosafety cabinet Class II.

Meaning of safety messages in procedures

Each safety message contained in the coming procedures starts with one of the following keywords. Their meaning is the following:

CAUTION! Indicates a dangerous situation which, if not avoided, may damage the sample.

***NOTICE**: Indicates obligations that optimize procedural success.*

***Note**: Emphasizes or adds information to the main text.*

Blood drawing

***Note**: Blood drawing may not occur at the same location where sample are processed. Below a few indications for proper blood drawing, transport and storage.*

Preliminary indications

Initial samples must be drawn prior to initiation of a therapy regimen. Subsequent samples can be drawn after the start of a therapy regimen, usually at three to four week intervals, to follow epithelial cell levels during therapy. If the patient is on doxorubicin therapy, allow at least seven days following administration of a dose of doxorubicin before blood draw.

Draw venous blood

CAUTION! Collect whole blood aseptically by venipuncture or from a venous port into a CellSave Preservative Tube only. Please refer to the *CellSave Preservative Tube Instructions for Use* for process, storage and handling instructions.

1. Fill the CellSave Preservative Tube until blood flow stops to ensure the correct ratio of sample to anticoagulant and preservative.
2. Immediately mix by gently inverting the CellSave Preservative Tube eight times to prevent clotting.

CAUTION! Inadequate or delayed mixing may result in inaccurate test results.

***NOTICE**: Discard the sample if clotted.*

3. Blood samples in CellSave Preservative Tubes should be stored or transported at room temperature. For optimal performance, use whole peripheral blood collected within the last 24 hours. **Do not refrigerate samples**.

Syringe preparation modes

***Note**: The syringe needs to be prepared when indicated in the procedure. A different preparation is required according to the type of aspiration required.*

Assemble the syringe

1. Take what follows:
    - a new 20 ml syringe from the CellMag Consumable box
    - the syringe holder and the spring stopper from the CellMag box
2. Move the syringe plunger back and forth for **five times** to keep it from seizing up during aspiration.
3. Insert the syringe in the syringe holder and then put the spring on the syringe plunger.
4. Press the spring slightly. Meanwhile, insert the spring stopper on the syringe plunger flange and the spring end from left to right.
5. Push the spring stopper in the syringe holder.

Prepare the syringe for complete aspiration

1. If the syringe is new, assemble it as described in "Assemble the syringe" on page 13.
2. Rotate the spring stopper clockwise until it locks. This is the **first position** to perform a complete aspiration.
3. Attach the cannula with extension tubing to the syringe and rotate the luer lock clockwise to lock it.

Prepare the syringe for limited aspiration

1. If the syringe is new, assemble it as described in "Assemble the syringe" on page 13.
2. Rotate the spring stopper clockwise until it locks. This is the **first position** to perform a complete aspiration.
3. Push and rotate the spring stopper clockwise until it locks. This is the **second position** to perform a limited aspiration.
4. Attach the cannula with extension tubing to the syringe and rotate the luer lock clockwise to lock it.

1. Sample preparation

A. Equilibrate reagents

- Remove all bottles from the foam insert and warm them at **room temperature** for at least **30 minutes**. The bottles of the Dilution Buffer and CellMag Buffer take longer to equilibrate to room temperature.
- Suggestion: mark the CELLSEARCH Conical Centrifuge tube (hereinafter "conical tube") according to the sample to be analyzed.

B. Prepare the blood sample

B1. Invert the CellSave Preservative tube **five times**.

B2. Transfer **7.5 ml** of blood into the conical tube provided.

B3. Using a new pipette add **6.5 ml** of Dilution Buffer into the conical tube.

B4. Close the conical tube using a conical tube cap provided and invert it **five times**.

B5. Use a **swing bucket centrifuge** to centrifuge the conical tube at **800 g for a full 10 min** in the BRAKE OFF mode.

B6. Analyze the tube to ensure that the red blood cell layer is separated from the plasma. Discard if there is no separation.

C. Add the reagents

C1. Open the conical tube and use a 10 ml pipette to aspirate the plasma. Leave about **3–4 ml** of plasma and set the cap aside for the following steps.

C2. Using a P1000 pipette carefully aspirate the remaining plasma. Leave at least **0.5–1 ml** of plasma.

C3. Add **3 ml** of CellMag Buffer (clear bottle with white cap) and **3 ml** of Dilution Buffer (amber bottles and caps).

C4. Close the conical tube and invert it **three times**.

C5. Open the conical tube and **set aside the cap** for the following steps.

C6. Add **150 µl** of Capture Enhancement Reagent (clear cap).

C7. Add **150 µl** of Anti-EpCAM ferrofluid (brown cap).

C8. Close the conical tube and invert it **three times**.

2. Magnetic separation

D. Enrich the target cells

CAUTION! During all magnetic incubations, do not rotate or remove the conical tube from CellMag.

D1. Insert the conical tube in CellMag and leave it in for **10 min** for magnetic separation.

D2. Remove the conical tube from CellMag and invert it **five times**.

D3. Repeat steps 1 and 2.

D4. Remove the conical tube cap and **discard it**.

D5. Mount the cannula guide on the conical tube.

D6. Put the conical tube in CellMag and leave it in for **20 min**. Meanwhile, prepare the provided syringe for a **complete aspiration** by locking the spring lock in **first position** (see "Prepare the syringe for complete aspiration" on page 13).

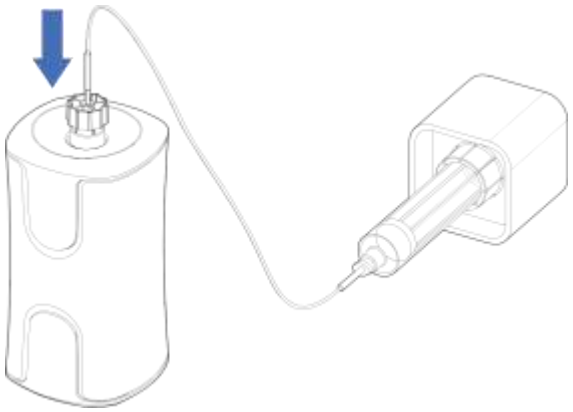
D7. At the end of the incubation, do not remove the conical tube from CellMag.

E. Aspirate the negative fraction

CAUTION! If the syringe plunger clogs during aspiration, move it gently up and down to unclog. If this does not work, take a new syringe and a new cannula with extension tubing and repeat the steps of "Syringe preparation modes" on page 13.

***Note**: For details, see the CellMag Consumable Instructions for Use.*

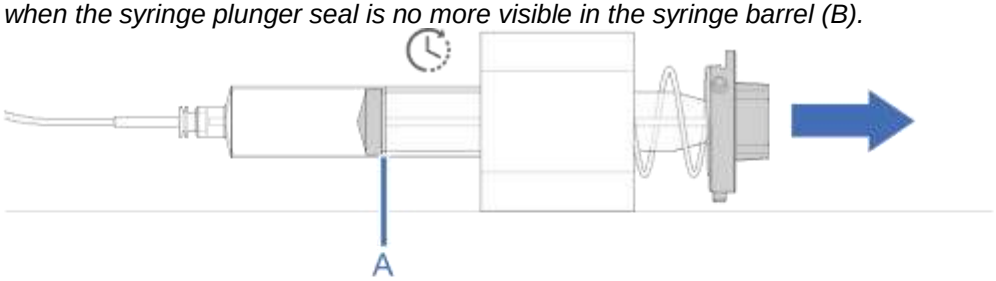
E1. Without removing the conical tube from CellMag, insert the cannula with extension tubing in the cannula guide until it touches the bottom of the tube.

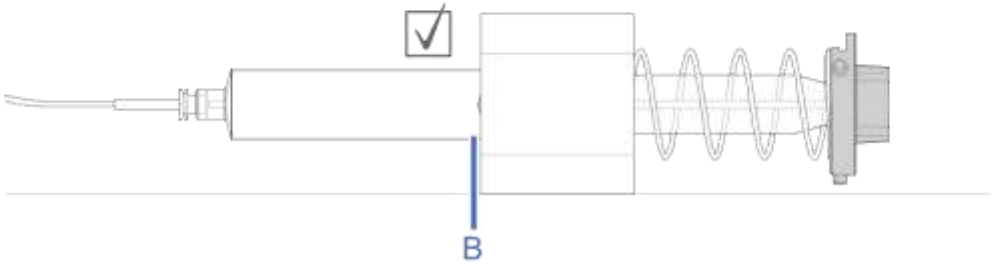


E2. Rotate the spring stopper counterclockwise to release the syringe plunger and aspirate the negative fraction.

CAUTION! Do not touch the spring stopper or the plunger while in motion.

***NOTICE**: Aspiration lasts a few minutes (A). Put the syringe on the bench during aspiration. The aspiration is completed when the syringe plunger seal is no more visible in the syringe barrel (B).*





E3. Remove the syringe from the syringe holder as follows:

If you want to...	Then...
disconnect the cannula with the extension tubing from the syringe	1. Rotate the luer lock counterclockwise to unlock the cannula. 2. Remove the syringe from the syringe holder.
keep the cannula with the extension tubing connected to the syringe	1. Remove the syringe from the syringe holder. 2. Pull the cannula through the slit on the syringe holder.

E4. Push the spring stopper to discard the aspirated negative fraction.

E5. Remove the conical tube from CellMag and put it in a rack.

E6. Remove the spring stopper, the spring and the syringe holder and **discard the syringe with cannula and extension tubing**.

3. Sample washing

F. Wash target cells

F1. Close the conical tube with a **new conical tube cap** and use **a swing bucket centrifuge** to centrifuge the conical tube at **300 g x 1 min**.

F2. Open the conical tube and **set aside** the conical tube cap for the following steps.

F3. Add **1.5 ml** of CellMag Buffer against the conical tube wall, ensuring to completely wash the sides of the tube.

F4. Repeat step 4 with **1.5 ml** of Dilution Buffer.

F5. Close the conical tube.

F6. Mix the conical tube with the vortex at **1600 RPM** for **10 s**.

CAUTION! If the vortex speed cannot be set, the speed should be high enough to wash the tube walls without damaging cells. Apply this process to all the next steps which require vortex.

F7. Open the conical tube and **set aside the conical tube cap** on a clean lint-free wipe for the following steps.

F8. Mount **a new cannula guide** on the conical tube.

F9. Put the conical tube in CellMag and leave it in for **10 min**.

F10. Prepare the syringe as described in "Prepare the syringe for complete aspiration" on page 13.

F11. At the end of the incubation, **do not remove** the conical tube from CellMag.

G. Aspirate the supernatant

CAUTION! If the syringe plunger clogs during aspiration, move it gently up and down to unclog. If this does not work, take a new syringe and a new cannula with extension tubing and repeat the steps of "Syringe preparation modes" on page 13.

G1. Insert a **new** cannula with extension tubing in the cannula guide until it touches the bottom of the tube.

G2. Rotate the spring stopper counterclockwise to release the syringe plunger and aspirate the supernatant.

CAUTION! Do not touch the spring stopper or the plunger while in motion.

***NOTICE**: Put the syringe on the bench during aspiration.*

G3. Remove the syringe from the syringe holder as follows:

If you want to...	Then...
disconnect the cannula with the extension tubing from the syringe	1. Rotate the luer lock counterclockwise to unlock the cannula. 2. Remove the syringe from the syringe holder.
keep the cannula with the extension tubing connected to the syringe	1. Remove the syringe from the syringe holder. 2. Pull the cannula through the slit on the syringe holder.

G4. Push the spring stopper to discard the aspirated supernatant.

G5. **Set aside the cannula and the extension tubing** on a clean lint-free wipe for the following steps.

G6. Remove the conical tube from CellMag.

4. Sample permeabilization and staining

H. Add the reagents

H1. Add the reagents **against the conical tube wall**, ensuring to completely wash the sides of the tube, and in the following order:
   1) **200 µl** of Dilution Buffer
   2) **150 µl** of Permeabilization Reagent (green cap)
   3) **150 µl** of Staining Reagent (white cap)
   4) **150 µl** of Nucleic Acid Dye (blue cap)
   5) **200 µl** of CellMag Buffer

H2. Resuspend the sample **five times** by gently pipetting.

H3. Close the conical tube and incubate it at **room temperature** for **20 min in the dark**.

I. Wash and enrich target cells

I1. Open the conical tube and set aside the conical tube cap for the following steps.

I2. Resuspend the sample **five times** by gently pipetting.

I3. Without touching the liquid located at the bottom of the conical tube, add the reagents against the wall of the conical tube in the following order:
   1) **1 ml** of CellMag Buffer
   2) **1 ml** of Dilution Buffer

I4. Resuspend the sample **five times** by gently pipetting.

I5. Mount the cannula guide on the conical tube.

I6. Put the conical tube in CellMag and leave it in for **15 min**. At the end of the incubation, do not remove the conical tube from CellMag. Meanwhile, prepare the syringe for **limited aspiration** by locking the spring stopper in **second position** (see "Prepare the syringe for limited aspiration" on page 13). Attach the cannula with extension tubing to the syringe and rotate the luer lock clockwise to lock it.

I7. Insert the cannula with extension tubing in the cannula guide until it touches the bottom of the tube.

I8. Rotate the spring stopper counterclockwise back to **first position**. This starts a limited aspiration that lasts approximately 20 seconds and only aspirates a small part of the sample.

I9. Remove the syringe from the syringe holder as follows:

If you want to...	**Then...**
disconnect the cannula with the extension tubing from the syringe	1. Rotate the luer lock counterclockwise to unlock the cannula. 2. Remove the syringe from the syringe holder.
keep the cannula with the extension tubing connected to the syringe	1. Remove the syringe from the syringe holder. 2. Pull the cannula through the slit on the syringe holder.

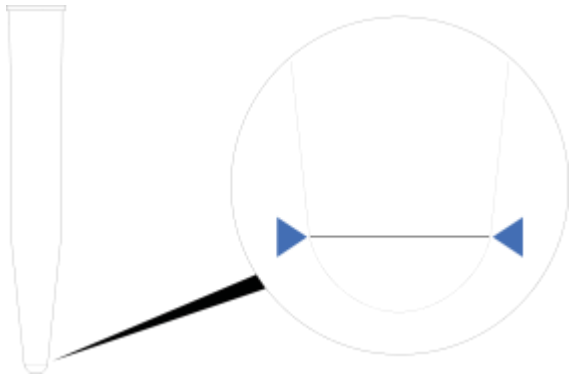
I10. Carefully extract the cannula with the extension tubing from the conical tube.

I11. Remove the cannula guide, the spring stopper, the spring and the syringe holder and discard the syringe, the cannula with the extension tubing and the cannula guide.

I12. Close the conical tube and use a swing bucket centrifuge to centrifuge it at **300 g x 5 min**.

I13. Open the conical tube and set aside the conical tube cap for the following steps.

I14. Using a P1000 pipette, aspirate the supernatant without touching the pellet. Leave around **100 µl** of sample or keep the liquid meniscus at the level of the mark on the bottom of the conical tube.



I15. Add **1 ml** of CellMag buffer and resuspend the sample **five times**.
I16. Close the conical tube and use a swing bucket centrifuge to centrifuge it at **300 g x 5 min**.
I17. Open the conical tube and set aside the conical tube cap for the following steps.
I18. Using a P1000 pipette, aspirate **1 ml** without touching the pellet.

5.Fixation

J. Add the fixative

J1. Add the reagents in the following order to the bottom of the conical tube **without touching** the tube walls:
   1) **150 µl** of Cell Fixative (red cap)
   2) **100 µl** of CellMag Buffer
J2. Close the conical tube and mix it with the vortex at **1600 RPM** for **10 s**.
J3. Incubate the conical tube at **room temperature** for **20 min** in the dark.
J4. If the next steps are performed later, store the tube at **4 °C in the dark for a maximum of 20 hours**.

6. Sample preparation for DEPArray

Introduction

Final sample preparation can be performed with or without VR NxT (the volume reduction instrument). Both methods are detailed below.

Before starting, thaw a 6 ml vial of DEPArray Buffer for fixed cells (hereinafter "DABUF") and keep it at **room temperature**.

***NOTICE**: Once thawed, the DABUF must be stored at 4 °C and used within 24 hours. Do not refreeze it.*

K. Sample preparation with VR NxT

K1. Open the conical tube and **set aside the conical tube cap** for the following steps.
K2. Add **0.25 ml** of DABUF to the sample without touching the walls or the liquid on the bottom of the conical tube.
K3. Close the conical tube and mix it with the vortex at **1600 RPM** for **10 s**.
K4. Use a **swing bucket centrifuge** to centrifuge the conical tube at **400 g x 10 min**.
K5. Open the tube gently and **set aside the conical tube cap** for the following steps.
K6. Using a P1000 pipette and without touching the cell pellet, follow the meniscus to carefully aspirate the supernatant. Leave around **100 µl** of it in the conical tube.
K7. Add **1 ml** of DABUF to the sample without touching the walls or the liquid on the bottom of the conical tube.
K8. Close the conical tube and mix it with the vortex at **1600 RPM** for **10 s**.
K9. Use a **swing bucket centrifuge** to centrifuge the conical tube at **400 g x 10 min**.
K10. Open the tube gently and **set aside the conical tube cap** for the following steps.

K11. Using a P1000 pipette and without touching the cell pellet, follow the meniscus to carefully aspirate the supernatant. Leave maximum **100 µl** of it in the conical tube.

K12. Using a P200 pipette and a low retention tip, without creating bubbles resuspend the sample and transfer it from the conical tube to the **MicroAmp Reaction tube 200 µl**.

K13. Using a P200 pipette and a low retention tip, without creating bubbles wash the conical tube with **50 µl** of DABUF and transfer the sample in the MicroAmp Reaction tube 200 µl previously used.

K14. Use a **swing bucket centrifuge with rotor for plates** and centrifuge it at **400 g x 10 min**: the sample is now ready for the volume reduction with VR NxT.

K15. Follow the instructions contained in the VR NxT Instruction Manual.

***Note**: Use the DEPArray IN program to reduce the sample volume.*

K16. At the end, resuspend the sample and load it in the DEPArray Cartridge.

L. Sample preparation without VR NxT

L1. Using a P1000 pipette and a low retention tip, transfer the whole sample into a **1.5 ml** protein low retention tube (sample tube) and resuspend it.

CAUTION! To minimize cell loss, **set aside the tip** for step 3.

L2. Add **0.25 ml** of DABUF into the conical tube and discard the tip.

L3. Using the same tip of step 1, wash the inner surface of the conical tube by gently pipetting the DABUF up and down **five times**.

L4. Transfer the sample back into the same sample tube. Discard the tip.

L5. Use a **swing bucket centrifuge** to centrifuge the sample tube at **400 g x 10 min**.

L6. Using a P1000 pipette, **without touching** the cell pellet follow the meniscus to carefully aspirate the supernatant. Leave maximum **50 µl** of it in the sample tube.

L7. Add **1 ml** of DABUF into the sample tube.

L8. Use a **swing bucket centrifuge** to centrifuge the sample tube at **400 g x 10 min**.

L9. Using a P1000 pipette, **without touching** the cell pellet follow the meniscus to carefully aspirate the supernatant. Leave maximum **100 µl** in the sample tube.

L10. Using a P200 pipette, **without touching** the cell pellet follow the meniscus to carefully aspirate the supernatant. Leave approximately **12 µl**.

L11. Using a P20 pipette and a low retention tip, check the volume of the liquid left in the sample tube and do what follows:

If the sample volume is...	**Then...**
lower than **12 µl**	adjust it to **12 µl** with DABUF.
greater than **12 µl**	1. Use a swing bucket centrifuge to centrifuge the sample tube at **400 g x 5 min**. 2. Carefully remove the extra volume using a P20 pipette.

L12. Resuspend the sample and load it in the DEPArray Cartridge.

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Instructions for Use

IFU_3003 - CellMag - Protocol and Reagents_Instructions for Use

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