FiberPrep®
DNA Extraction Kit

DNA Extraction Protocol – 10 Extractions prior to Easy Comb service

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Reference: EXT-001-10EC_IFU_001_WW_EN_1

GENOMIC VISION

CONTENT

1. INTRODUCTION

This FiberPrep® DNA Extraction kit has been specifically designed to provide high quality DNA solutions for Molecular Combing carried out by Genomic Vision. Molecular Combing is Genomic Vision's proprietary technology used for many applications and particularly for studying:

- DNA replication: physical characteristics and kinetic parameters of DNA replication can be studied by monitoring the incorporation of modified nucleotides in growing cells.
- Large genetic rearrangements: by use of specific labeled probes, large rearrangements (from 1 kb to several Mb) can be detected. This includes duplications, deletions, contractions or expansions and CNVs generally, as well as balanced translocations, inversions, etc.

The DNA is then stretched onto silanized coverslips (COV-002, Genomic Vision, Paris, France) using the FiberComb® Molecular Combing System (MCS-001, Genomic Vision, Paris, France). Several hundreds of single DNA molecules are stretched in a parallel way and with a constant stretching factor, which enables the correspondence between a physical measure and the DNA length (1 µm = 2 kb). The hybridization and detection steps are followed by scanning with the FiberVision® (SCN-001, Genomic Vision, Paris, France) or FiberVisionS® (SCN-002, Genomic Vision, Paris, France) automated scanner and analysis with the dedicated FiberStudio® software (FSE-LSR-P1, Genomic Vision, Paris, France).

2. INTENDED USE & PRINCIPLE OF PROCEDURE

The FiberPrep® DNA Extraction kit is intended for the extraction of DNA from fresh blood samples, purified peripheral blood mononuclear cells (PBMC) or cultured eukaryotic cell lines for Molecular Combing applications carried out by Genomic Vision. This kit is designed to purify and store high molecular weight DNA (average size: 400 kb), by protecting them from mechanical stress. After embedding cells in an agarose plug, proteins are digested by a proteinase and cell membranes are solubilized by a surfactant under inhibition of DNase activity. The treated DNA plugs are then stored in the storage Buffer and ready to be shipped at Genomic Vision for the preparation of a high molecular weight DNA solution.

3. STORAGE CONDITIONS

The complete kit is received and then stored at +2°C to +8°C. Each element is necessary for the entire process of extraction before sending of the plugs.

4. PRODUCT USE LIMITATIONS

For general laboratory use.

5. KIT REAGENTS AND STORAGE CONDITIONS

- The FiberPrep® DNA Extraction kit contains:

Buffers	Volume in the kit	Storage temperature	Condition after opening/receipt
Buffer 2 (Plug Buffer)	1 mL	+2°C to +8°C	Stable for 1 month after opening Do not melt and refrigerate more than 10 times
Buffer 3 (Proteinase Buffer)	2,5 mL	+2°C to +8°C	N/A
Buffer 4 (Washing Buffer)	5,5 mL	+2°C to +8°C	N/A
Buffer 5 (Storage Buffer)	28 mL	+2°C to +8°C	N/A
Component 3 (Proteinase)	300 µL	+2°C to +8°C	N/A
Individual bag with Buffer 1 (Suspension Buffer)	500µL	+2°C to +8°C	Stable for 1 month after receipt

* Buffer 1 is only used for extraction of DNA from cultured cells. If you extract your DNA from blood, please replace Buffer 1 by standard cell culture grade PBS (Phosphate-Buffered Saline).

The FiberPrep® DNA Extraction Kit contains reagents to perform 10 extractions, preparing the plugs independently or by several (by 2, 3 or 10 plugs at the same time for example).

6. SAFETY PRECAUTIONS AND WARNINGS

- Wear a suitable lab coat, disposable gloves and protective goggles when handling reagents and samples. Thoroughly wash hands before and after handling them.
- Do not pipette by mouth.
- Samples and reagents of human origin as well as contaminated material and products must be discarded in a contaminated residue container.
- If liquid containing human material is spilled, clean the affected area with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

Some compounds are associated with safety pictograms:

Compounds	Pictograms	Hazard statements*	Precautionary statements*
Buffer 1		H317 H334	P261 P272 P280 P285 P302 + P352 P304 + P341 P321 P333 + P313 P342 + P311 P363 P501
Buffer 3		H319	P264 P280 P305 + P351 + P338 P337 + P313
Buffer 5		H373	P260 P314 P501
Component 3		H315 H319 H334 H335	P261 P264 P271 P280 P285 P302 + P352 P304 + P340 + P312 P305 + P351 + P338 P332 + P313 P337 + P313 P342 + P311 P362 P403 + P233 P405 P501

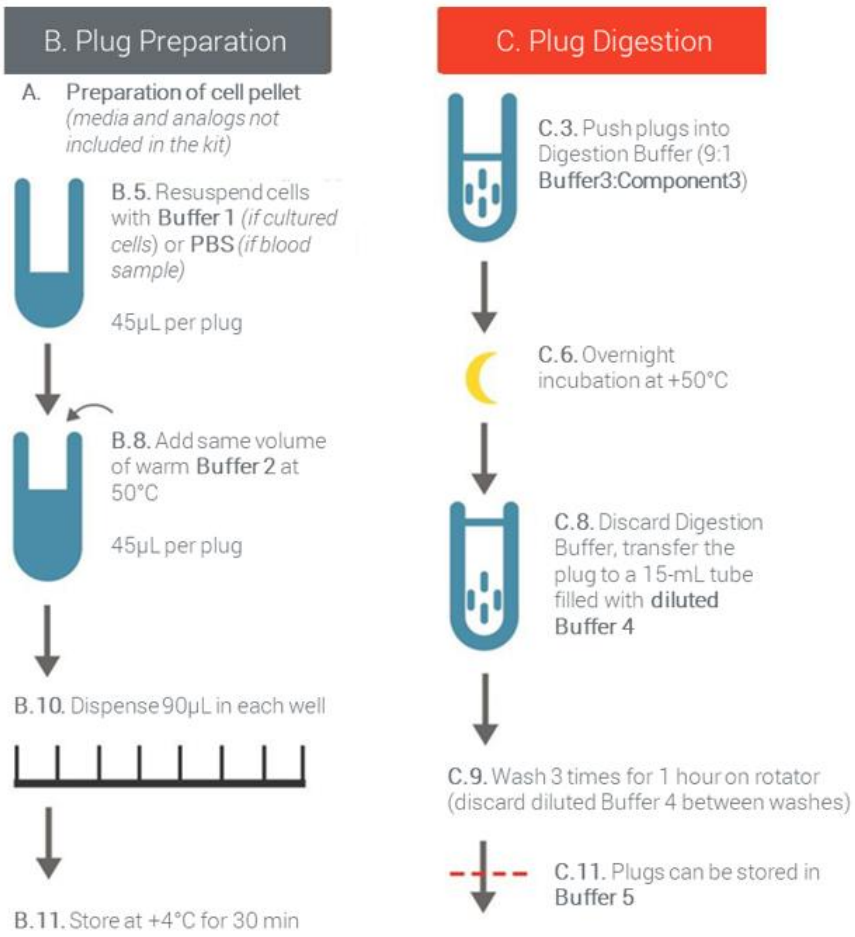
*****See the details of the statements in the appendix.*

For more information, please consult the appropriate material safety data sheets (MSDS) on our website: www.genomicvision.com.

7. ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED

Materials	Supplier	Reference
Gel plug Mold	BioRad	1703713
Test tube rotator	Any	-
Waterbath	Any	-
Cell culture CO_2 incubator	Any	-
Counting Chamber	Any	-
Falcon tube 15mL and 50mL	Any	-
2mL Round-bottom centrifuge tube	Any	-
Cell culture flask	Any	-
Centrifuge	Any	-
PBS	Any	-
Screened caps	BioRad	1703711

8. ASSAY PROCEDURE



8.1 Preparation of cells (Step A)

Before starting cell manipulation:

- Set two water baths at +68°C and +50°C.
- Melt the Buffer 2 of FiberPrep® kit at +68°C for 10 min. Check that the gel is completely melted. Homogenize the melted solution by inverting the tube and keep it at +50°C until use.

For *cultured cells*, harvest your cells according to cell lines' specifications. The cells can be adherent or in suspension.

Respect safety measures associated with the type of cells you are manipulating. Cells should be considered intact until Digestion treatment of cells is completed.

For *blood samples*, best results are obtained with white blood cells purified by either red blood cell lysis or Ficoll® gradient centrifugation. Blood should be fresh or have a maximum storage time of 5 days at 4°C. Tested anticoagulants are EDTA, ACD. This procedure is not validated for extraction from frozen blood.

8.2 Plug preparation: Embedding cells into agarose plugs (Step B)

⚠ *Caution: The generation of cell suspension and the subsequent casting of the plugs should be performed as rapidly as possible in order to minimize premature cell lysis*

The number of cells to be embedded in a plug depends on the downstream application:

- For replication studies using approximately 10,000 cells / plug is recommended.
- For physical cartography (hybridization) applications, typically 500,000 – 1,000,000 cells / plug is recommended.
- Optimal cell number has to be decided by users for their own applications.

1. Count cell number of your cell suspension with a Kovas® slide and a microscope or another equivalent.
2. Centrifuge the appropriate quantity of cells suspension at 160g for 5 min at room temperature.

Tip: Due to pipetting loss and/or errors, we recommend to perform the experiment with a dead volume corresponding to 0,5 extra plug until plugging.

3. Decant the supernatant of cell suspension gently. Remove remaining supernatant by pipette as much as possible.
4. Calculate the appropriate volume of **Buffer 1** (45 µL is needed for 1 plug).

Tip: Continue to count 0,5 extra plug because of pipetting loss and/or errors.

Example: For the preparation of 5 plugs, use 247.5 µL (= 5.5 x 45 µL) of the cell suspension.

5. Completely suspend the cell pellet with the appropriate volume of **Buffer 1** (for cultured cells) or **PBS** (for cells extracted from blood).

⚠ *Caution: Store the Buffer 1 at +4°C for maximum 1 month after receipt.*

6. Homogenize well by pipetting (10 times up and down).

⚠ *Caution: It is important that any clumps of cells have been removed prior to embedding cells into the agarose plug.*

7. Warm the cell suspension to +50°C for 10 seconds.
8. Mix the suspension with the same volume of **melted Buffer 2** (identical to the volume previously calculated for Buffer 1) kept at +50°C as indicated in step A.
9. Homogenize the solution well at +50°C by pipetting (10 times up and down).

⚠ *Caution: Make sure that the cell/Buffer 2 mix is homogeneous, and no clumps are visible.*

10. Immediately dispense the mixture quickly into DNA plug molds. The volume of one well is approximately 90 µL. Keep the solution in the water bath while dispensing and do not hesitate to homogenize between each distribution by pipetting up and down.

⚠ *Caution: Do not allow bubbles to form.*

11. Set the DNA plug mold horizontally at +4°C (in a box to prevent the agarose gel from desiccating) for 30 min.

Tip: If the plugs are not well solidified or look too soft, incubate them 15 min longer at +4°C.

8.3 Protein digestion treatment of plugs (Step C)

1. Prepare 250 µL of complete **Protease digestion buffer** (**Buffer 3** + **Component 3**, 9:1 volume) per

plug. Plugs from the same condition may be incubated together. Use a 15 mL-tube for up to 3 plugs, or a 50 mL-tube for more than 3 plugs and up to 10 plugs.

⚠ *Caution: This mix cannot be prepared in advance*

Example: For 10 plugs, add 250 µL of Component 3 to 2250 µL of Buffer 3.

2. Push the solidified plug out of the mold into the **Digestion Buffer** using a plastic plunger.

Tip: Place the plugs on the surface of the tube and slide them up to the digestion buffer to prevent them from being damaged by falling violently into the solution.

3. Warm up the tube containing the plug(s) at +50°C. Since the tubes contain small volumes of solution, make sure the tubes are held vertically in the water bath.
4. After 30 min at +50°C, gently swirl the tubes to homogenize the solution.
5. Keep the tubes at +50°C overnight (16-18 hours).
6. The following day, dilute enough **Buffer 4** in a 1:100 proportion with nuclease-free water for the following three washing steps. If possible, autoclave **diluted Buffer 4** in advance or use only recently diluted Buffer. Do not store it.

   According to the number of plugs, use 15 mL-tube (for up to 3 plugs) or 50mL-tube (for up to 10 plugs).

Example:

Type of tube	*15 mL-tube*	*50 mL-tube*
Buffer 4 volume	*0,5 mL*	*1,7 mL*
Nuclease-free water	*49,5 mL*	*168,5 mL*
Total volume	*50 mL (for 3 washes)*	*170 mL (for 3 washes)*

7. Transfer DNA plugs to appropriate tube(s) filled with **diluted Buffer 4** (max 3 plugs/15 mL-tube and 10 plugs/50 mL-tube) with a spatula. Be careful when manipulating the plugs.

Tip: Make sure there is a small air bubble in the tube, to allow a soft flow of liquid. If the bubble is too big, it may damage the plugs, if it is too small, it may get stuck in the tube. Check the movement of the bubble after putting the tube on the test tube rotator.

8. Wash the plugs for 1 hour on a test tube rotator.
9. Change the **diluted Buffer 4** and wash again for 1 hour with rotation. Repeat twice (in total 3 washes).

Tip: Screened caps can be useful for efficiently recovering the plug and removing the washing buffer.

10. Transfer the plugs independently in 2 mL-round-bottom microtubes completely filled with Buffer 5.
11. Ship the plugs (in the tubes) to Genomic Vision at +4 °C.

9. APPENDIX

Hazard statements details

H317	May cause an allergic skin reaction
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled
H319	Causes serious eye irritation

H373	May cause damage to organs (Respiratory Tract) through prolonged or repeated exposure if inhaled
H315	Causes skin irritation
H335	May cause respiratory irritation

Precautionary statements details

P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray
P264	Wash skin thoroughly after handling
P271	Use only outdoors or in a well-ventilated area
P272	Contaminated work clothing should not be allowed out of the workplace
P280	Wear protective gloves/ eye protection/ face protection
P285	In case of inadequate ventilation wear respiratory protection
P302 + P352	IF ON SKIN: Wash with plenty of soap and water
P304 + P340 + P312	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell
P304 + P341	IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P314	Get medical advice/ attention if you feel unwell
P321	Specific treatment (see supplemental first aid instructions on this label
P332 + P313	If skin irritation occurs: Get medical advice/ attention
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention
P337 + P313	If eye irritation persists: Get medical advice/ attention
P342 + P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor
P362	Take off contaminated clothing and wash before reuse
P363	Wash contaminated clothing before reuse
P403 + P233	Store in a well-ventilated place. Keep container tightly closed
P405	Store locked up
P501	Dispose of contents/ container to an approved waste disposal plant

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Intellectual Property Rights

This product or the use of this product is subject to proprietary rights (EP2007/059299, IB2009/007197). The Molecular Combing technology and products are covered by patents (FR2716206, FR 2716263, FR 2737574, FR 2755149) owned by Genomic Vision S.A.

Quality Control

The FiberPrep® DNA Extraction Kits undergo strict Quality Controls performed in Genomic Vision's laboratories. Should you nevertheless experience problems with the product, please contact the technical support team.

Trademarks:

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