**For Research Use Only**
**Not for use in diagnostic procedures**

# HEMAgene®·BUFFY COAT

## For the ambient temperature stabilization of high molecular weight DNA

- Fresh or frozen buffy coat samples
- Fresh or frozen white blood cell pellet samples

# Protocol handbook

REF **HG-BCD-50**

**DNA stabilizing reagent sample pack**

REF **HG-BCD-250**

**DNA stabilizing reagent**

⚠ 15°C 30°C

Wash with water if liquid solution comes in contact with eyes or skin. Do not ingest. See MSDS at www.dnagenotek.com. Patent (www.dnagenotek.com/legalnotices)

*Superior samples*
*Proven performance*

Made in Canada
DNA Genotek Inc.
Ottawa, ON, Canada K2K 1L1
Subsidiary of OraSure Technologies, Inc.

DNA genotek
www.dnagenotek.com

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## Kit contents*

| | Reagent sample pack | Reagent solution |
|---|---|---|
| Catalogue # | HG-BCD-50 | HG-BCD-250 |
| HEMAgene•BUFFY COAT DNA stabilizing reagent | 50 mL | 250 mL |
| Number of 0.5 mL buffy coat preparations | 10–30 | 50–160 |

* Kit may contain either 50 mL (HG-BCD-50) or 250 mL (HG-BCD-250) bottles.

# Storage

HEMAgene®•BUFFY COAT DNA stabilizing reagent (HG-BCD) should be stored at room temperature (15°C-30°C) and is stable until the "use by" date indicated on the bottle label.

## Intended use

HG-BCD is intended for the ambient temperature stabilization of high molecular weight DNA in:

- Fresh or frozen buffy coat samples
- Fresh or frozen white blood cell pellet samples

This liquid-based stabilizer is suitable for ambient temperature shipping and room temperature storage of buffy coat and white blood cell pellet samples. White blood cell pellets are defined as buffy coat samples which have been depleted of red blood cells via a red blood cell lysis step.

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## Safety information

HG-BCD does not contain toxic chemicals. Blood component specimens and laboratory chemicals should be handled with appropriate biosafety practices in accordance with your standard laboratory procedures. It is recommended to wear a laboratory coat, disposable gloves, and protective goggles.

For more information, please refer the appropriate material safety data sheet (MSDS) available at www.dnagenotek.com.

## Quality control

In accordance with DNA Genotek's ISO 13485:2003 certified Quality Management System, each lot of HG-BCD is tested against defined specifications to ensure a consistent high quality product.

## Equipment to be supplied by user

- 15 mL conical centrifuge tubes for initial sample processing
- suitable tubes for storage of the HEMAgene•BUFFY COAT sample

  **Note:** HEMAgene•BUFFY COAT sample can be stored as a single multi-use sample or as multiple smaller aliquot samples.
- pipettes and pipette tips
- vortex
- 0.9 % NaCl if preparing white blood cell pellet samples

## Protocol guidance

- Buffy coat may vary in leukocyte concentration depending on the number of nucleated cells in the original whole blood sample and the method of buffy coat preparation.
- HG-BCD is flexible and can be scaled up for samples with high leukocyte counts

When stabilizing a buffy coat from a whole blood sample an important factor in optimizing the concentration of the resultant sample is adding the appropriate ratio of HEMAgene•BUFFY COAT DNA stabilizing reagent (HG-BCD). This ratio is always determined by the original blood draw volume of the sample being collected. Regardless of the original draw volume, it is common to harvest 500 µL of buffy coat for processing but it is important to note that the concentration of cells within this 500 µL aliquot will vary depending on the original blood draw volume. In the table to the right the recommended ratios and brief protocols for varying draw volumes are listed. A full step-by-step protocol follows after this table.

**Note:** A "buffy coat preparation" is defined as either a 500 µL buffy coat or a white blood cell pellet that has been resuspended with 500 µL of NaCl.

| Whole blood draw volume | Buffy coat preparation: HG-BCD ratio recommendation | Volumes for a 500 µL buffy coat preparation |
|---|---|---|
| 3–4 mL whole blood | 1:3 | **4 mL whole blood draw into EDTA blood tube**<br>i. 0.5 mL buffy coat preparation from ~4 mL whole blood.<br>ii. Add 1.5 mL HG-BCD reagent and vortex to lyse and stabilize the sample.<br>iii. Store at room temperature until required for processing. |
| 5–6 mL whole blood | 1:5 | **6 mL whole blood draw into EDTA blood tube**<br>i. 0.5 mL buffy coat preparation from ~6 mL whole blood.<br>ii. Add 2.5 mL HG-BCD reagent and vortex to lyse and stabilize the sample.<br>iii. Store at room temperature until required for processing. |
| 7–8 mL whole blood | 1:7 | **8 mL whole blood draw into EDTA blood tube**<br>i. 0.5 mL buffy coat preparation from ~8 mL whole blood.<br>ii. Add 3.5 mL of HG-BCD reagent and vortex to lyse and stabilize the sample.<br>iii. Store at room temperature until required for processing. |
| 9–10 mL whole blood | 1:9 | **10 mL whole blood draw into EDTA blood tube**<br>i. 0.5 mL buffy coat preparation from ~10 mL whole blood.<br>ii. Add 4.5 mL HG-BCD reagent and vortex to lyse and stabilize the sample.<br>iii. Store at room temperature until required for processing. |

## Protocol 1 for the stabilization of DNA
## From fresh or frozen buffy coat samples

If starting with a frozen buffy coat sample, thaw at 37°C for 2 minutes or until the sample is completely thawed.

### Procedure

| Sample preparation steps | Notes |
|---|---|
| 1. Transfer 0.5 mL of the buffy coat sample to a 15 mL conical tube. | |
| 2. Add appropriate volume of HG-BCD reagent to buffy coat according to table on page 5. | • Ratio of buffy coat to HG-BCD depends on starting blood draw volume (see page 5). |
| 3. Vortex vigorously for 15 seconds to ensure complete mixing and lysis of the sample. | • In cases where it is difficult to pipette the sample, an additional 1 mL – 5 mL of HG-BCD can be added to the sample. Vortex again to reduce sample viscosity if necessary. |
| 4. Store the sample with any of the following storage options:<br>• Store at room temperature in any size aliquot(s).<br>• Freeze in any size aliquot(s). | • Samples can be stored as a larger multi-use sample or in multiple smaller volume aliquots as desired.<br>• The HEMAgene•BUFFY COAT sample can withstand multiple freeze-thaw cycles without DNA degradation or loss. Cryovials are suggested for freezing long-term. |

## Protocol 2 for the stabilization of DNA
## From fresh or frozen white blood cell pellet samples

If starting with a frozen white blood cell pellet sample, thaw at 37°C for 2 minutes or until the sample is completely thawed.

### Procedure

| Sample preparation steps | Notes |
|---|---|
| 1. Add 0.5 mL of 0.9% NaCl to the white blood cell pellet to create a suspension. | |
| 2. Transfer the 0.5 mL suspension to a 15 mL conical tube. | |
| 3. Add appropriate volume of HG-BCD reagent to buffy coat according to table on page 5. | • Ratio of buffy coat to HG-BCD depends on starting blood draw volume (see page 5). |
| 4. Vortex vigorously for 15 seconds to ensure complete mixing and lysis of the sample. | • In cases where it is difficult to pipette the sample, an additional 1 mL – 5 mL of HG-BCD can be added to the sample. Vortex again to reduce sample viscosity if necessary. |
| 5. Store the sample with any of the following storage options:<br>• Store at room temperature in any size aliquot(s).<br>• Freeze in any size aliquot(s). | • Samples can be stored as a larger multi-use sample or in multiple smaller volume aliquots as desired.<br>• The white blood cell pellet HEMAgene•BUFFY COAT sample can withstand multiple freeze-thaw cycles without degradation or loss. Cryovials are suggested for freezing long-term. |

## Shipping

Once the sample has been prepared with HG-BCD, the HEMAgene•BUFFY COAT sample can be shipped at ambient temperature without the need for dry ice. The sample is stable at 50°C for up to 2 weeks and can withstand multiple freeze-thaw cycles during transport.

**Technical support is available Monday to Friday (9h00 to 17h00 EST):**

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com

Australian Sponsor
Emergo Australia, Level 20, Tower II, Darling Park, 201 Sussex Street, Sydney, NSW 2000 Australia

Some DNA Genotek products may not be available in all geographic regions.
HEMAgene is a registered trademark of DNA Genotek Inc.
All DNA Genotek protocols, white papers and application notes are available in the support section of our website at www.dnagenotek.com.

**Label legend:**

| | |
|---|---|
| | Use by |
| REF | Catalog number |
| ⚠ | Caution, consult instructions for use |
| 15°C 30°C | Storage instructions |
| | Manufacturer |

PD-HB-00003 Issue 5/2014-09