



## SAMPLE APPLICATION INSTRUCTION MANUAL

PREPARATION AND APPLICATION OF  
BLOOD PRODUCTS, DMSO OR GLYCEROL  
BACTERIAL STOCKS TO GENPLATES  
FOR DNA STORAGE

Technical Support  
9AM-5PM - US Pacific Time  
+1-888-GEN-0755  
[www.genvault.com](http://www.genvault.com)



### BLOOD PRODUCT APPLICATION PROTOCOL

For the application of whole blood and buffy coat samples to GenVault GenPlates.

**Before applying buffy coat, please refer to *Recommended Buffy Coat Preparation* (p. 3).**

**For optimal recovery of DNA, please refer to the *Blood Sample Handling Guidelines* (p. 7).**

1. Remove the **GenPlate** from the foil pouch only when you are ready to use it.  
*The foil pouch is reusable for storing or shipping the plate.*
2. Remove and discard the temporary adhesive seal on the GenPlate before applying the sample to the storage element.  
*The GenPlate should be used within 60 minutes of removal from foil pouch or kept in a cool, dry environment (ambient temperature and <40% relative humidity).*
3. Bring your sample to room temperature and rock for 10-15 minutes. Spot 10  $\mu$ l of the sample in the center of each well.  
*It is not necessary for the pipette tip to contact the storage matrix, but the sample must be entirely dispensed onto the storage matrix to engage bactericidal and virucidal agents.*
4. After applying the sample (or samples to a multi-sample plate), place the unsealed plate in a biosafety hood or a GenVault Drying Station (<40% humidity) overnight (12-16 hours).
5. After samples are dry, apply the **Adhesive Storage Plate Seal**. Ensure that the seal is firmly attached to the top of the plate; avoid creating bubbles on the surface of the plate.
6. Plate is now ready for storage.
7. To achieve maximum DNA yield, allow the plate to cure for an additional 2 weeks before recovering DNA.

## RECOMMENDED BUFFY COAT PREPARATION

Please read before proceeding with *Blood Product Application Protocol* (p. 2).

### WHAT YOU NEED

- ANTICOAGULANT-TREATED BLOOD
- 1 – 15 ML CONICAL TUBE PER SAMPLE

- FICOLL-PAQUE™ PLUS

GE HEALTHCARE – PRODUCT CODES 17-1440-02 & 17-1440-03

- SALT SOLUTION

SEE FICOLL-PAQUE PLUS PROTOCOL FOR PREPARATION INSTRUCTIONS

1. Mix together 2 ml of anticoagulant-treated whole blood and 2 ml of salt solution.
2. Mix the Ficoll-Paque Plus well and add 3 ml of it to a 15 ml conical tube.
3. **Carefully** add the 4 ml of whole blood plus salt solution on top of Ficoll-Paque Plus layer in the 15 ml tube. **Do not mix the two layers.**
4. Centrifuge sample at 400 x g for 30-40 minutes at 18-20°C. *Upon completion, 4 layers should be visible (From top to bottom: plasma, buffy coat, Ficoll-Paque Plus, and granulocytes & erythrocytes).*
5. Draw off the plasma layer using a clean Pasteur pipette and discard. Be careful not to disturb the buffy coat layer.

6. Using a clean pipette, transfer the buffy coat layer into a clean 15 ml conical tube. Be sure not to remove any Ficoll.
7. Add 6 ml of salt solution to the conical tube containing the buffy coat, and mix it up and down.
8. Centrifuge the conical tube for 3 minutes at 2000 x g. A pellet should form.
9. Aspirate the salt solution and discard.
10. Re-suspend the pellet in 400 µl 1x TE and proceed directly to the ***Blood Product Application Protocol*** (p.2). *One 2 ml sample of whole blood will fill 40 GenPlate elements with buffy coat.*

---

### Note

The pellet can be resuspended in as little as 200 µl to increase the quantity of DNA stored on a single element, but will result in only 20 GenPlate elements spotted with buffy coat.

---

## DMSO OR GLYCEROL BACTERIAL STOCKS APPLICATION PROTOCOL

For the application of DMSO or Glycerol Bacterial Stocks **ONLY** to GenVault Whole blood GenPlates.

1. Remove the **GenPlate** from the foil pouch, *only* when you are ready to use it. It will have a silver foil seal.  
*The foil pouch is reusable for storing or shipping the plate.*
2. Remove and discard the temporary silver foil seal on the GenPlate before applying DMSO or Glycerol Bacterial Stocks to the storage element.  
*GenPlate should be used within 60 minutes of removal from foil pouch or kept in a cool, dry environment (ambient temperature and <40% relative humidity).*
3. Spot 10 µl of DMSO or Glycerol Bacterial Stocks to the center of each well. Typically DMSO Stocks are 10% DMSO. Glycerol stocks may be 50% down to 8% glycerol. Depending on the downstream application for the DNA recovered from the glycerol stocks, the one ml of stock may be spun down to a cell pellet resuspended in 100 µl LB, TB, SOC, TE, or TBS for a 10-fold concentration and then spotted on the GenPlate.  
*It is not necessary for the pipette tip to contact the storage matrix, but the sample must be entirely dispensed onto the storage matrix to ensure its preservation and proper recovery.*
4. After applying the sample (or samples to a multi-sample plate), place the unsealed plate in a biosafety hood or a GenVault Drying Station (<40% humidity) overnight (12-16 hours).
5. After samples are dry, apply the **Adhesive Storage Plate Seal**. Ensure that the seal is firmly attached to the top of the plate; avoid creating bubbles on the surface of the plate.
6. Plate is now ready for storage.

## BLOOD SAMPLE HANDLING GUIDELINES

Please read before proceeding with **Blood Product Application Protocol (p.2)**.

### ROOM TEMPERATURE BLOOD STORAGE PRIOR TO SPOTTING

- **0-24 HOURS** – No change in DNA yield observed.
- **24-72 HOURS** - Acceptable for use with GenVault system, but a slight decrease in yield may be observed.
- **72 HOURS-8 DAYS** - A steady decline in DNA yield is observed.
  - **5 DAYS** - 20% drop in yield
  - **8 DAYS** - 33% drop in yield
- **2-8 WEEKS** - Not recommended for use with GenVault system. A considerable decline in the amount of recoverable DNA observed.

### 4°C BLOOD STORAGE PRIOR TO SPOTTING

- **0-24 HOURS** - No change in DNA yield observed.
- **24 HOURS-8 DAYS** - Acceptable for use with GenVault system, but a slight decrease in yield may be observed.
- **2-8 WEEKS** - Not recommended for use with GenVault system. A considerable decline in the amount of recoverable DNA observed.

### FROZEN BLOOD STORAGE PRIOR TO SPOTTING

- **LOW TEMPERATURE (-20°C)** - Results in an immediate drop in DNA yield compared to storage at 4°C. This decline progresses steadily to a 30-40% drop in DNA recoverability after 1 year.
- **ULTRA LOW TEMPERATURE (-80°C)** – There is no influence on DNA yield or quality with long term storage (years). However, short term storage (24 hours) will alter DNA size.



## REFERENCES

Visvikis S, Schlenck A, Maurice M. DNA extraction and stability for epidemiological studies. *Clin Chem Lab Med.* 1998. **36**(8):551-5.

Madisen L, Hoar DI, Holroyd CD, Crisp M, Hodes ME. DNA banking: the effects of storage of blood and isolated DNA on the integrity of DNA. *Am J Med Genet.* 1987. **27**(2):379-90.

Schunemann HJ, Stanulla M, Trevisan M, Aplan PD, Freudenheim JL, Muti P. Short-term storage of blood samples and DNA isolation in serum separator tubes for application in epidemiological studies and clinical research. *Ann Epidemiol.* **10**:538-544.

Ross KS, Haites NE, Kelly KF. Repeated freezing and thawing of peripheral blood and DNA in suspension: effects on DNA yield and integrity. *J Med Genet.* 1990. **27**(9):569-70.

Adams M, Lee TH, Busch MP, Heitman J, Marshall GJ, Gjerset GF, Mosley JW. Rapid freezing of whole blood or buffy coat samples for polymerase chain reaction and cell culture analysis: application to detection of human immunodeficiency virus in blood donor and recipient repositories. The Transfusion Safety Study Group. *Transfusion.* 1993. **33**(6):504-8.

Farkas DH, Drevon AM, Kiechle FL, DiCarlo RG, Heath EM, Crisan D. Specimen stability for DNA-based diagnostic testing. *Diagn Mol Pathol.* 1996. **5**(4):2.