

HbF Diluent

Cat. No. 9341

HbF Diluent is intended for use as a diluent when quantitating levels of hemoglobin F greater than 10% by radial immunodiffusion.

SUMMARY

The Helena HbF QUIPlate system is an excellent method for measurement of HbF in whole blood. It provides rapid quantitation and needs no reagent preparation and storage or expensive instrumentation.

To accurately quantitate HbF levels greater than 10%, the patient sample must be diluted. Since the HbF result must be multiplied by a dilution factor of 10, even a very low amount of HbF in the diluent blood sample will contribute significantly to the final patient result.

Helena responded to the need for an appropriate diluent by developing the HbF Diluent which consists of chromatographically purified hemoglobin A (HbA). Since the Diluent is completely free of HbF, it ensures that only HbF in the patient sample contributes to the quantitation. It provides greater accuracy and precision in the results of those samples with HbF values greater than 10%.

PRINCIPLE

The patient sample is adjusted to 1 g/dL total hemoglobin. It is mixed with the HbF Diluent in a 1:10 dilution in order to obtain a value that falls within the standard curve.

REAGENT

HbF Diluent contains purified HbA which has been lyophilized and reconstituted in purified water with 0.1% sodium azide added as a preservative. The HbA concentration in the Diluent is 1 g/dL.

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST

The human blood used in this product has been found negative for Hepatitis antigen (HBsAg) and HIV antibody; however, the diluent should be handled with the same precautions as those observed with patient samples. To prevent the formation of toxic vapors, sodium azide should not be mixed with acidic solutions. When discarding reagents containing sodium azide, always flush sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are highly explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

Preparation for Use: The diluent is ready for use as packaged but **should be vortexed before use**. No adjustment of the total hemoglobin is needed.

Storage and Stability: The diluent should be stored at 2 to 6°C and is stable until the expiration date indicated on the label.

Signs of Deterioration: The product should not be used if it shows signs of cloudiness or particulate matter which may be an indication of deterioration or microbial contamination.

SPECIMEN COLLECTION AND HANDLING

Refer to Helena Laboratories' HbF QUIPlate Procedure, Pro. 43.

PROCEDURE

1. Determine the total hemoglobin concentration of the patient whole blood sample (collected in EDTA) using a standard laboratory method.
2. Prepare a 1 g/dL hemoglobin dilution of the patient sample as directed in the HbF QUIPlate Procedure No. 43.
3. After dilution, agitate samples to ensure complete hemolysis.
4. Using the 1 g/dL patient sample preparation, make a 1:10 dilution using 1 part (5 µL) patient sample and 9 parts (45 µL) HbF Diluent (HbA level in the diluent is 1 g/dL as packaged). Vortex both the Diluent and the sample preparation before pipetting them to prevent any settling effects.
5. Mix the dilution well.
6. Apply 5 µL diluted sample to the HbF QUIPlate.
7. Complete the procedure by applying 5 µL of HbF Standards and additional patient samples to the plate. Incubate the plate 24 hours at room temperature, measure the precipitin rings and determine the HbF% as outlined in the HbF QUIPlate Procedure(Pro 43).
8. Multiply the value obtained from the standard curve by 10 (dilution factor) to obtain the patients HbF quantitation.

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LIMITATIONS

1. Adequate vortexing of the specimens before dilution is necessary for reproducibility.
2. The Helena HbF QUIPlate produces accurate results from whole blood samples with HbF levels above 0.5%. Samples with HbF levels greater than 10% must be diluted with purified HbA Diluent.
3. Patient precipitin rings should compare to the standard rings in sharpness & intensity. On rare occasions, a patient may have a very faint diffuse ring indicative of a contaminant. Some patients who have less than 0.5% or no HbF may exhibit no precipitin ring at all.

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run studies were performed using a dilution tested 10 times on one plate. The results were as follows:

$$n = 10 \quad \bar{X} = 71.7 \\ SD = 2.8 \\ CV\% = 3.9$$

Between Day studies were performed on 5 samples. A fresh dilution of each was made and tested in duplicate on two plates for five days yielding 20 data points for each of the five samples. The results were as follows:

Donor 1: n = 20	Mean 80.6% 1 S.D. 5.2% CV 6.5%	Donor 4: n = 20	Mean 84.2% 1 S.D. 4.3% CV 5.1%
Donor 2: n = 20	Mean 81.1% 1 S.D. 6.0% CV 7.4%	Donor 5: n = 20	Mean 86.0% 1 S.D. 2.6% CV 3.1%
Donor 3: n = 20	Mean 76.8% 1 S.D. 2.9% CV 3.8%		

COMPARISON

- 1) Comparison studies were performed on 27 cord blood samples utilizing the RID procedure and electrophoresis. The results of the Paired- t Test showed a test statistic of 3.534 which is greater than the critical value for t of 2.06, thus rejecting the null hypothesis and proving the correlation.
- 2) A linear regression equation was also calculated using these cord bloods plus 5 Sudden Infant Death samples with intermediate levels of HbF. The results were as follows:

$$X = \text{electrophoresis} \\ Y = \text{RID} \\ \text{Slope} = 0.8899 \\ \text{Intercept} = 4.591 \\ r = 0.8912$$

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