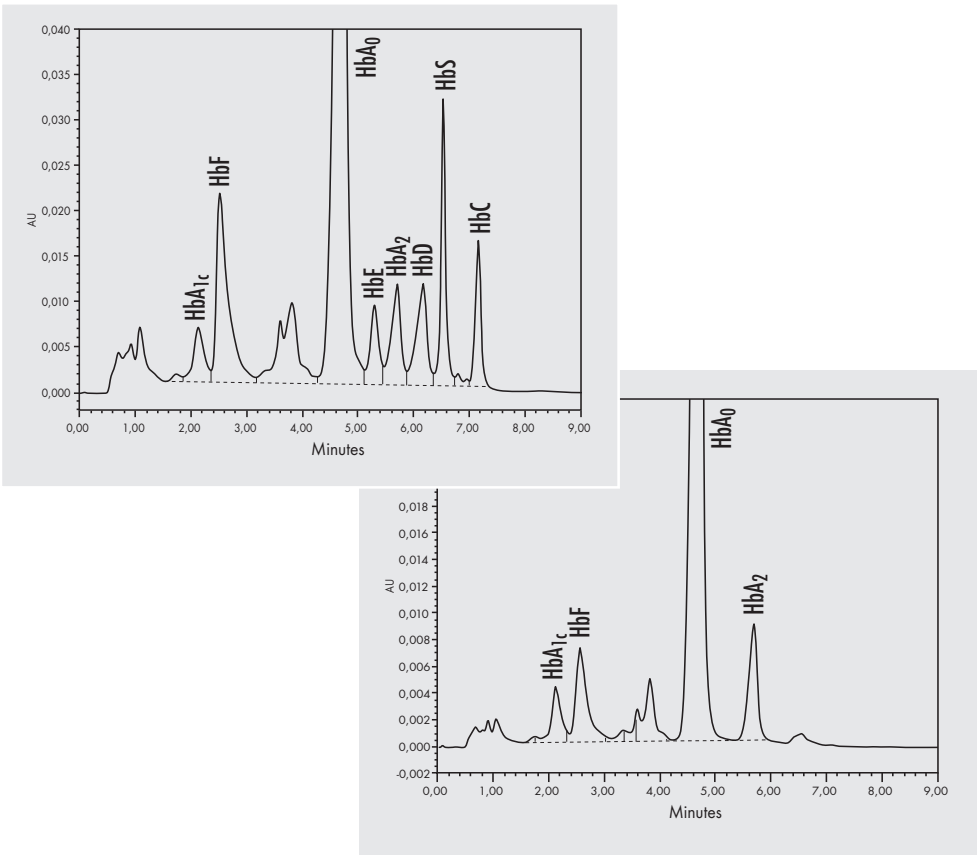
Hemoglobin Variants

Reagent Kit for HPLC Analysis

> All important hemoglobin variants within 9 min
> Improved sample throughput and efficiency



Hemoglobin (Hb) consists of four polypeptide chains called globins and one heme molecule bound to each globin. Different genetic defects may lead to abnormalities of hemoglobin: structural defects, thalassaemic syndromes and the hereditary persistence of foetal Hb. Abnormal hemoglobin shows decreased function of different severity and, depending on the type of hemoglobinopathy, tendencies towards promoting vaso-occlusion (clogging of blood vessels) by erythrocytes. Among the most severe clinical expressions are syndromes such as sickle cell anaemia, hypochromic anaemia and familial cyanose. These syndromes may be caused by different mutations and an adequate therapy requires the exact identification of the hemoglobinopathy. Some of these will not be sufficiently eliminated by symptomatic therapy such as blood transfusion, but require laborious approaches such as bone marrow transplantation. However, carriers of the genetic defects may minimise the manifestation of the disease by an adapted lifestyle, thus an early diagnosis, especially for children, is of paramount importance.

The Chromsystems reagent kit for the HPLC analysis of hemoglobin variants in whole blood allows for a fast sample preparation and a powerful and reproducible separation of all major abnormal hemoglobins such as HbS, HbC, HbE and HbD as well as markers for thalassemia such as HbA_2 and HbF. No interfering peaks are detected. The total analysis is performed on a binary HPLC gradient system (high or low pressure) within 9 minutes by means of a UV/VIS detector.

Specifications

Linearity:	up to at least 75 % (HbA_2)
	up to at least 70 % (HbF)
Limit of quantification:	2 % (HbA_2)
	1 % (HbF)
Intraassay:	CV < 8 %
Interassay:	CV < 4 %
Recovery:	100 % (HbA_2)
	99.2 % (HbF)
Analysis time:	9 min

Pre-Analytic Treatment

Specimens: whole blood, can be transported without cooling.
Stability of specimens: at room temperature approx. 24 h, at +2 to +8 °C up to 36 h, for longer periods freeze and store below -18 °C. Once thawed, samples should not be refrozen.

Sample Preparation

→ Dilute 5–8 µl capillary whole blood with 1 ml Hemolysis Reagent, mix well.
→ Inject 10–20 µl of the hemolysis mixture into the HPLC system.

Samples prepared as above can be stored at room temperature for about 24 hours, up to 36 hours at +2 to +8 °C, frozen below -18 °C up to 3 months.

HPLC Parameters

A binary HPLC gradient system with UV detection is required.

Injection volume:	10–20 µl
Flow rate:	1.5 ml/min
Wavelength:	415 nm
Column temperature:	ambient (~ 25 °C)

Ordering Information

These products are not available in the USA

Order no.	*Product*
15330	Reagent kit for the HPLC analysis of Hemoglobin Variants For 1000 analyses, HPLC column included
	Components available separately:
15331	Buffer A, 2.1 l
15331/C	Buffer A, 5.0 l
15332	Buffer B, 2.8 l
15332/C	Buffer B, 5.0 l
15334	Hemolysis Reagent, 500 ml
15335	Wash Buffer, 1.0 l
15390	HPLC column, equilibrated, with test chromatogram
	Startup Accessories:
15007/B	Reaction Vials, 1000 pcs.
55033	PEEK prefilter, 5 µm, 5 pcs.
15010	PEEK prefilter housing, 1 pc.
	Note: *HPLC system gradients should be adapted and optimized depending on the relevant void volumes.* *Contact our specialists for assistance.*
	Also available for Hemoglobin analysis:
15440	Reagent kit for the HPLC analysis of β-Thalassemia
15100	HPLC column for HbA1c analysis, ask for detailed information