

# Thromboplastin L

REF 5265HL  
REF 5265L  
REF 5267L

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe

Helena Biosciences Europe, Queensway South, Team Valley Trading Estate, Gateshead, Tyne and Wear, NE11 OSD, United Kingdom

Tel: +44 (0)191 482 8440

Fax: +44 (0)191 482 8442

Email: [info@helena-biosciences.com](mailto:info@helena-biosciences.com)

Web: [www.helena-biosciences.com](http://www.helena-biosciences.com)

HL-2-3035P 2015/10 (1)

## Thromboplastin L

The Thromboplastin L kit is intended for carrying out clot based haemostasis assays.

The first standardised one-stage prothrombin time test was developed by Dr. Armand Quick in 1935. It has now become the basic coagulation screening test for the diagnosis of congenital and acquired deficiencies of clotting factors from the extrinsic pathway (factors II, V, VII and X)<sup>1,2</sup>. It is also used for the induction and monitoring of oral anticoagulant therapy<sup>3,4</sup> and can be used to assess the protein synthesis capability of the liver in chronic or acute hepatic disorders. Thromboplastin L is of rabbit brain origin but resembles human preparations in its low International Sensitivity Index (ISI). The ISI of Thromboplastin L is approximately 1,1 and is calibrated against the WHO international reference preparation<sup>5</sup>. Thromboplastin L is particularly suited to the monitoring of oral anticoagulant therapy and, in conjunction with the appropriate factor deficient plasma, the measurement of factor activity in the extrinsic pathway. Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal citrated plasma, the clotting mechanism is activated, leading to a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency.

### WARNINGS AND PRECAUTIONS

The reagents contained in this kit are for *in vitro* diagnostic use only – DO NOT INGEST. Wear appropriate personal protective equipment when handling all kit components. Refer to the product safety declaration for the link to appropriate hazard and precautionary statements where applicable. Dispose of components in accordance with local regulations.

### COMPOSITION

Composition	Contnt	Description	Préparation
Thromboplastin L	2 x 5 mL (REF 5265HL) 8 x 5 mL (REF 5265L) 10 x 10 mL (REF 5267L)	Liquid Rabbit Brain Thromboplastin containing Calcium Chloride, stabilisers and preservatives.	The liquid, calcified thromboplastin is ready-for-use. No further calcium is required to carry out standard PT Assays. The contents of the vial should be mixed well before use. (5 minutes on roller).

Each kit contains Instructions For Use.

Each kit contains lot specific reference values insert.

### ITEMS REQUIRED BUT NOT PROVIDED

The below products can be used in conjunction with Thromboplastin L:

REF 5519 ISI Calibrant Plasma Set  
REF 5490 INR Reference Set

### STORAGE, SHELF-LIFE AND STABILITY

Unopened reagents are stable until the given expiry date when stored under conditions indicated on the vial or kit label.

Thromboplastin L Opened vials are stable for 2 months at \*2 – 8°C, 5 days at \*15°C (on-board Sysmex CA-1500 ) and 6 hours at \*37°C (on-board AC-4 including reagent container and cap). A shift-use stability of 7 days (Sysmex CA-1500) can be achieved. DO NOT FREEZE. Large clumps of particles or changes in expected values may indicate product deterioration.

### SAMPLE COLLECTION AND PREPARATION

Plastic or siliconised glass should be used throughout. Blood (9 parts) should be collected into 3.2% or 3.8% sodium citrate anticoagulant (1 part). Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at \*18 –24°C. Testing should be completed within 4 hours of sample collection, or plasma can be stored frozen at -20°C for 2 weeks or -70°C for 6 months. Thaw quickly at \*37°C prior to testing. Do not keep at \*37°C for more than 5 minutes<sup>6</sup>.

### PROCEDURE

For accurate INR reporting, it is recommended to determine the laboratory specific ISI of the reagent with the testing system in use. The Helena Biosciences Europe ISI Calibrant Plasma Set (REF 5519) is recommended for this purpose<sup>7,8</sup>. This should be performed for each new reagent batch. The Helena Biosciences Europe INR Reference Set (REF 5490) should be used to check for shifts in the local system ISI which have been noted with changes in laboratory temperature and post instrument servicing, amongst other local variations.

#### Manual Method

- Mix sufficient Thromboplastin L to complete the anticipated testing for the day and incubate at \*37°C for no more than 4 hours.
- Pre-warm 0.1 mL of the test plasma at \*37°C for 2 minutes.
- Add 0.2 mL of freshly mixed thromboplastin reagent to the plasma while simultaneously starting a stopwatch.
- Note the time for clot formation to the nearest 0.1 seconds.

#### Automated Method

Refer to the appropriate instrument operator manual for detailed instructions or contact Helena Biosciences Europe for instrument specific application guides.

### INTERPRETATION OF RESULTS

Results should be reported to the nearest 0.1 seconds and duplicates should agree within 5% of each other. %PT values can be interpolated from the calibration graph (%PT of PT Calibration plasmas versus measured clot time), which should be a straight line when plotted on log-log graph paper.

INR values can be calculated using the following formula: 



 
INR
=
(
P
T
Time
Patient

/

Mean
Normal
P
T
Time

)

I
S
I




{\displaystyle \ \mathrm {INR} = (PT\ Time\ Patient / Mean\ Normal\ PT\ Time)^{ISI}}

For clear guidance on the indications for and management of patients on warfarin, please refer to The British Society for Haematology, for their most current edition of 'Guidelines on oral anticoagulation with warfarin'. At time of printing this is the 2011 fourth edition<sup>9</sup>.

### LIMITATIONS

The use of serial dilutions of a reference plasma for the %PT curve is not recommended as this can lead to discrepancies caused by the low fibrinogen in the reference plasma dilutions which are not reflected in patient samples having predominantly normal fibrinogen levels. Helena Biosciences Europe advise use of the 5504R %PT/Direct INR kit for this purpose.

### QUALITY CONTROL

Each laboratory should establish a quality control program. Normal and abnormal control plasmas should be tested prior to each batch of patient samples, to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid.

Helena Biosciences Europe supplies the following controls available for use with this product:

REF 5186 Routine Control N  
REF 5187 Routine Control A  
REF 5183 Routine Control SA  
REF 5490 INR Reference Set

### REFERENCE VALUES

Reference values can vary between laboratories depending on the techniques and systems in use. For this reason each laboratory should establish its own reference ranges. This is particularly important for local ISI calibration. Using the Sysmex series of instruments, normal values ranging from 11.50 - 14.60 seconds; 0.930 - 1.160 INR; 79.10 - 112.80 %PT are typical.

### PERFORMANCE CHARACTERISTICS

The following performance characteristics have been determined by Helena Biosciences Europe or their representatives using a Sysmex CA-1500 coagulation instrument. Each laboratory should establish its own performance data.

Reproducibility					
Sample	Routine Control N	Routine Control A	Routine Control SA	SD	CV (%)
Repeatability	0.07	0.59	0.24	1.09	0.45
Between-run	0.10	0.83	0.16	0.75	0.49
Between-day	0.04	0.32	0.06	0.27	0.25
Within-device / Laboratory	0.12	1.07	0.29	1.35	0.72

#### Interferences

Helena Thromboplastin L is insensitive to Heparin levels of up to 2 U/mL. Using a 5% interference threshold, there is no significant interference from Haemoglobin at concentrations up to 10 g/L. Using a 5% interference threshold, there is no significant interference from Bilirubin at concentrations up to 0.5 g/L for Thromboplastin L. Lipid interference testing demonstrates that lipid levels do not directly affect the clot time of the reagent up to 3.75g/L. Lipid concentrations in excess of this prevent clot detection.

#### Method Comparison

Comparison of clot time in seconds and INR values were determined using Thromboplastin L and Thromboplastin LI on 268 samples. The following correlations were obtained:

Thromboplastin L (Seconds) = 0.9911x + 0.1038 




r

2


=
0.9941


{\displaystyle r^{2}=0.9941}

 



n
=
268


{\displaystyle n=268}

Thromboplastin L (INR) = 0.9853x + 0.0261 




r

2


=
0.9500


{\displaystyle r^{2}=0.9500}

 



n
=
268


{\displaystyle n=268}

### BIBLIOGRAPHY

- Quick AJ (1935) A Study of the Coagulation Defect in Hemophilia and Jaundice. *Am. J. Med. Sci.* **190**: 501.
- Biggs R (1976) Human Blood Coagulation, Haemostasis and Thrombosis, 2nd Edition, Blackwell Scientific Publications, London.
- Hirsh J, Poller L, Deykin D, Levine J, Dalen JE

