Axis-Shield Heparin Binding Protein EIA

REF FMHBP100IUO

Σ 96

FOR INVESTIGATIONAL USE ONLY. THE PERFORMANCE CHARACTERISTICS OF THIS PRODUCT HAVE NOT YET BEEN ESTABLISHED.

Read highlighted changes, revised October 2017

INTENDED USE

This kit is for INVESTIGATIONAL USE ONLY. Do not report out any patient sample results generated from the use of this kit.

PRINCIPLE OF THE ASSAY

The microtitre break-apart wells are coated with a monoclonal antibody to Heparin Binding Protein (HBP). During the first incubation, HBP in the sample specifically binds to the antibody-coated surface. The wells are then washed to remove unbound components. In the second incubation the Conjugate binds to any captured HBP. After further washing the bound HBP is detected by incubation with the Substrate. Addition of the Stop Solution terminates the reaction, resulting in a coloured end-product. The concentration of HBP in ng/mL is directly related to the colour generated and can be estimated by interpolation from a dose-response curve based on Calibrators.

KIT COMPONENTS

SAMPLE DIL	1 x 20.0 mL	Phosphate buffered saline, 2% protein stabiliser, 0.05% (v/v) Tween 20, 0.1% (w/v) Proclin. **READY TO USE. NB: WARNING.**
SOLN STOP	1 x 15.0 mL	Sulpuric acid 0.25 mol/L aqueous solution. **READY TO USE. NB: DANGER**
BUF WASH 10 X	3 x 25.0 mL	Phosphate buffered saline, 0.55% (v/v) Tween 20. **Dilute 1/10 before use.**
SUBS	1 x 15.0 mL	3,3',5,5'-Tetramethylbenzidine, buffer solution. **Do not expose to light during storage** **READY TO USE. NB: WARNING.**
CONJ	1 x 15.0 mL	Horseradish peroxidase-labelled monoclonal antibody to HBP, 0.1% (w/v) p-Hydroxyphenylacetic acid, 0.15% (w/v) Proclin and 1% protein stabiliser (w/v) in HEPES buffer. **READY TO USE. NB: WARNING.**
CONTROL L CONTROL M CONTROL H	1 x 0.3 mL 1 x 0.3 mL 1 x 0.3 mL	Recombinant Heparin Binding Protein in human plasma and buffer, 0.05% (w/v) Proclin **Dilute 1/40 with Sample Diluent before use, as for samples.** **NB: WARNING.**
CAL A - CAL F	6 x 1.0 mL	Recombinant Heparin Binding Protein in buffer, 0.01% (w/v) Proclin, 0, 12.5, 25, 50, 100 and 200 ng/mL **READY TO USE. NB: WARNING.**
MTP 8x12	8 x 12 Well microtitre strips	Coated with Heparin Binding Protein monoclonal antibody, in a re-sealable foil pack with desiccant. Individual wells can be broken off from each microtitre strip.

STANDARDISATION

There is currently no internationally recognized reference method or reference material for standardization. The Axis-Shield Heparin Binding Protein EIA Calibrators are traceable to internal reference standards which underwent a one-time value assignment.

STORAGE OF REAGENTS

Opened (In-Use) Kit Stability

A kit was opened, and reused on three occasions over a three month period with no adverse effect on kit performance. Following use, components must be returned to storage at 2-8°C.

Unopened kit stability

At 2-8°C unopended components are stable until as directed on the labels.

Handling and Procedural Notes

1. Store kit components at 2-8°C and use until the expiry date on the labels Do not use expired reagents.
2. Do not mix different lot numbers of kit component.
3. Do not freeze kits.
4. The Wash Buffer Concentrate and the three kit controls must be diluted before use. All other reagents are ready-to-use.
5. Diluted Wash Buffer can be stored at 2-8°C for up to 1 week.
6. Replace surplus (unused) microtitre strips in the foil pack with the desiccant. Ensure seal is integral and return to 2-8°C, until required.
7. Do not expose Substrate to light during storage.
8. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.

Indications of Deterioration

The Substrate should be colourless to very pale blue in colour. Any colour may indicate contamination and the reagent must be discarded. Turbidity or precipitation in any component indicates deterioration and the component should be discarded.

Sample Collection and Storage

- The assay is recommended for human **citrated plasma only.** It is the responsibility of the operator to verify that the correct tube type is used.
- Whole blood must be separated within 2 hours (preferably within one hour).
- **Haemolysed samples must not be used.**
- Thoroughly mix thawed samples before assay.
- Plasma samples can be stored at 2-8°C or -70°C for up to 1 week.
- Avoid more than 1 freeze/thaw cycle.
- Samples should only be diluted **immediately** prior to use.

WARNINGS AND PRECAUTIONS

For Investigational Use Only. The characteristics of this product have not yet been established. Procedures must be followed and no patient sample results should be reported.

Safety Precautions

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2.  The controls contain human plasma tested by FDA-cleared assays for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HIV-1/HIV-2 and anti-HCV or HCV RNA, and found to be non-reactive/negative. As no known test offers complete assurance that infectious agents are absent, controls should be considered potentially infectious and handled with the same precautions as any other potentially biohazardous material.[1,2] Clinical and Laboratory Standards Institute (CLSI) approve guidelines M29-A3.[3]
3. Do not pipette by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
5. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
6. Material safety data sheets for all components are available on request from Axis-Shield Diagnostics Ltd.

 WARNING **Sample Diluent, Conjugate, Control L, M, H, Calibrator A- F**	**WARNING** H317 - **PREVENTION** P272 - P280 - P363 -	 May cause an allergic skin reaction. Contaminated work clothing should not be allowed out the workplace. Wear protective gloves/protective clothing/eye protection/face protection. Wash contaminated clothing before use.
 WARNING **SUBSTRATE**	**WARNING** H302 – H312 – H315 – H319 – H332 – H335 – **PREVENTION** P260 – P271 – P280 – **RESPONSE** P301+310 – P304+340 – P305+351+338 P312 -	 Harmful if swallowed. Harmful in contact with skin. Causes skin irritation. Causes serious eye irritation. Harmful if inhaled. May cause respiratory irritation. Do not breathe dust/fume/gas/mist /vapours spray. Use only outdoors or in a well-ventilated area. Wear protective gloves/protectiveclothing/eye protection/face protection. IF SWALLOWED : Immediately call a POISON CENTRE or doctor / physician. IF INHALED : remove victim to fresh air and keep at rest in a position comfortable for breathing. IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. Call a POISON CENTRE or doctor/physician if you feel unwell.
 **DANGER** **Stop Solution**	**WARNING** H314 – **PREVENTION** P260 – P280 – **RESPONSE** P301+330+331 – P303+361+353 – P304+340 – P305+351+338 –	 Causes severe skin burns and eye damage. Do not breathe dust/fumes/gas/mist /vapours spray. Wear protective gloves/protective clothing/eye protection/face protection. IF SWALLOWED: rinse mouth. Do NOT induce vomiting. IF ON SKIN (or hair):Take off immediately all contaminated clothing. Rinse skin with water/shower. IF INHALED : Remove person to fresh air and keep comfortable for breathing. IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

PREPARATION

Materials/Equipment Required but not Provided

1. 96 well plate/strip reader with 450nm filter.
2. Precision pipette(s) to dispense 10 µL, 100 µL, 390 µL. Automatic Pipette to dispense 100 µL. Automatic pipette to dispense 300 µL for manual washing. Automatic plate washer optional.
3. Disposable pipette tips.
4. Glass/plastic measuring cylinder for dilution of the Wash Buffer Concentrate.
5. Distilled/deionised water.
6. Paper towels.
7. Timer for 10 and 60 minute intervals.

Preparation for the Assay

Allow all kit components, including the microtitre strips, to warm up to 18-25°C for 30-60 minutes before use. Mix reagents by gentle inversion.

NB – The Wash Buffer Concentrate can precipitate (crystals can be visible) when stored at 2-8°C, if this occurs allow the wash buffer to warm up until **NO** precipitation is evident to the naked eye (can be placed in an incubator at 37°C if required to speed the process).

Dilute the following components and mix thoroughly:

Components	Volume	Add
Wash Buffer Concentrate	1 Vial (25mL)	225 mL distilled/deionised water
Kit Controls/samples	10 µL	390 µL Sample Diluent

Calculate the number of microtitre strips required for the current assay and retain these in the microtitre strip holder. Return surplus strips to the resealable foil pack with the desiccant and store at 2-8°C until required. Ensure that all strips are securely held within the microtitre strip holder. Users may wish to number each strip along the top edge to aid identification. Retain the microtitre strip holder for future use.

ASSAY PROTOCOL

1. Reference wells for identification.
2. Pipette 100 µL Calibrators, pre-diluted (1/40) kit controls and patient samples in duplicate into appropriate wells. Remember to change pipette tips between additions. This step should not exceed **10 minutes** for any one set of Calibrators / controls / samples.
3. Incubate for **60 minutes** at 18-25°C.
4. Decant strip contents by quick inversion over a sink suitable for the disposal of biological materials, bearing in mind the potential infectious hazard of the controls and samples.
5. Wash wells **three times** with a minimum of 300 µL diluted Wash Buffer per well i.e. ensure wells are completely filled with wash buffer. **Decant after each wash step and after final wash, blot inverted strips well with paper towels.**
6. Add 100µL Conjugate to each well.
7. Incubate for **60 minutes** at 18-25°C.
8. Repeat steps 4 and 5.
9. Add 100 µL Substrate to each well.
10. Incubate for **10 minutes** at 18-25°C. **Do not decant.**
11. Add 100 µL Stop Solution to each well, in the same order and at the same rate as the Substrate. Tap wells gently to mix.
12. Read strips at 450nm. **This should not exceed 4 hours following the addition of the Stop Solution.**

QUALITY CONTROL

Ensure that adequate maintenance and calibration of the plate-reader is performed according to the manufacturer's instructions, and that a wavelength of 450nm is employed.
User should ensure they are fully acquainted with the instructions for the assay, particularly the Warnings and Precautions section and the Handling and Procedural Notes. It is recommended that the Kit Controls are run in duplicate in all assays to monitor the quality of the test procedures. Failure of any Control to meet the Control specifications below renders the assay invalid. The operator may repeat the assay, having reviewed their procedure. Laboratories may wish to include in-house controls in each assay run:

Control	Specification
Low Control expected range	<12 ng/mL
Medium Control expected range	14.9 – 34.7 ng/mL
High Control expected range	59.6 – 139.2 ng/mL

For technical assistance contact the manufacturer.

LIMITATIONS OF USE

- FOR INVESTIGATIONAL USE ONLY. The characteristics of this product have not yet been established.
- Do not report out any patient sample results from the use of this kit.

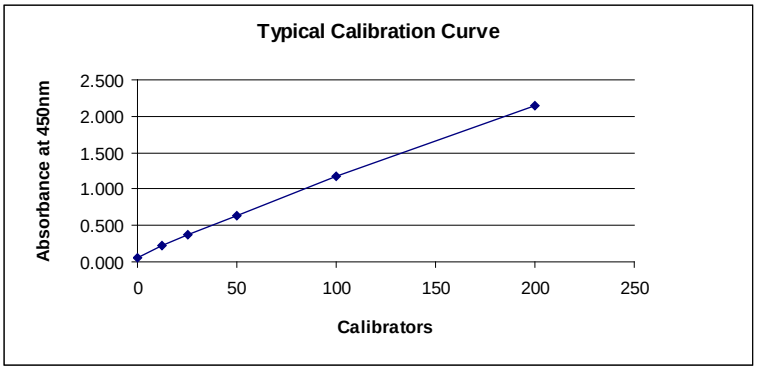
RESULTS

Unit of Measure

The unit of measurement for the Axis-Shield Heparin Binding Protein EIA is ng/mL. Do not report out any patient sample results generated from the use of this kit.

CALCULATION AND INTERPRETATION OF RESULTS

Consider each assay separately when calculating and interpreting results.
Plot the mean absorbance value of each Calibrator against the Calibrator concentrations on suitable graph paper. If using a plate reader with internal software plot the mean absorbance values of the Calibrators on the y-axis versus the units in ng/mL on the x-axis using a cublic spline, linear regression, point-to-point or 4PL curve fit algorithm.
Note – Log-Logit should **not** be used for this assay.
Concentrations of Controls and samples can then be read from the Calibration curve; a typical plot is shown below for reference purposes, it must not be used for interpreting results.



Cross-reactivity

A study was performed based on guidance from the CLSI Document EP7-A2.[4] No cross-reactivity was observed for the following cross-reactants (1µg/mL); Human Neutrophil Elastase, Proteinase 3 and Cathepsin G.

Interference

A study was performed based on guidance from the CLSI document EP7-A2.[4] Potentially interfering substances were evaluated to determine whether HBP concentrations were affected when using the Axis-Shield Heparin Binding Protein EIA. The substances listed below were spiked into samples with HBP concentrations across the assay range. The samples were assayed, and the HBP concentrations of the spiked samples were compared to reference samples. No interference as defined by either a Unit change of ≤ 2ng/mL or a percent difference of ≤ 10% was observed for the potentially interfering substances at the concentrations presented in the following table.

Potential Interfering Substance	No interference at the following concentration
Total Protein	73.2 mg/mL
Triglyceride (Intralipid Solution)	8.5 mg/mL
Bilirubin	0.2 mg/mL

Drug interferences

A study performed based on guidance from the CLSI document EP7-A2.[4] Potentially interfering drugs were evaluated to determine whether HBP concentrations were affected when using the Axis-Shield Heparin Binding Protein EIA. The drugs listed below were spiked into samples with HBP concentrations across the assay range. The samples were assayed, and the HBP concentrations of the spiked samples were compared to reference samples. No interference as defined by either a Unit change of ≤ 2ng/mL or a percent difference of ≤ 10% was observed for the potentially interfering drugs at the concentrations presented in the following table.

Drug	No interference at the following concentration
Imipenem	1.18 mg/mL
Cefotaxime	0.9 mg/mL
Vancomycin	3.5 mg/mL
Penicillin G	0.4 mg/mL
Dopamine	0.145 mg/mL
Noradrenaline	2 µg/mL
Dobutamine	11.2 µg/mL
Furosemide	0.02 mg/mL
Heparin	16.3 IU/mL

REFERENCES

1. World Health Organization. *Laboratory Biosafety Manual* 3rd ed.Geneva: World Health Organization; 2004.
2. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories.* 5th ed. Washington, DC: US Government Printing Office; December 2009.
3. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition.* CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
4. Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition*. CLSI Document EP7-A2. Wayne, PA: CLSI: 2005.

KEY TO SYMBOLS

REF	Catalogue number	CONTROL L	Low Control
LOT	Lot	CONTROL M	Medium Control
Σ 96	96 tests	CONTROL H	High Control
	Caution	CONJ	Conjugate
	Consult instructions for use	SAMPLE DIL	Sample Diluent
	Protect from Light	SUBS	Substrate
	Use by	SOLN STOP	Stop Solution
+2°C +8°C	Store at 2-8°C	BUF WASH 10 X	Wash Buffer
	Manufactured by	MTP 8 x 12	Microtitre (Breakapart) Strips
		CAL A - CAL F	Calibrators A to F

Axis-Shield Diagnostics Ltd.,
The Technology Park, Dundee,
DD2 1XA, UK
Tel: +44 (0) 1382 422000
Fax: +44 (0) 1382 422088
Web: www.axis-shield.com

RPBL1064/R3 Ver: 2017/10