BioVendor Group

ENG

Instructions for Use:

VASOACTIVE INTESTINAL POLYPEPTIDE ELISA

Catalogue number:

RCD033R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1. INTENDED USE

For the quantitative measurement of Vasoactive Intestinal Polypeptide (VIP) in human EDTA plasma by an ELISA (Enzyme-Linked Immunosorbent Assay).

This kit is intended for research use only and is not to be used for any diagnostic procedures.

2. INTRODUCTION

The VIP ELISA is a two-step competitive immunoassay.
In the first incubation step, competition occurs between VIP present in calibrators, controls, specimen samples and a biotin-labelled antigen (biotin conjugate) for a limited number of anti-VIP antibody binding sites on the microplate wells.
Excess and unbound materials are removed by a washing step.
In the second incubation step, streptavidin-HRP conjugate is added, which binds specifically to any bound biotin conjugate. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of VIP present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the colour from blue to yellow. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of VIP in specimen samples and controls can be directly read.

3. PRECAUTIONS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:

– Do not pipette by mouth.
– Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
– Wear protective clothing and disposable gloves.
– Wash hands thoroughly after performing the test.
– Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged, do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.

8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. When adding deionized water for the reconstitution of lyophilized components, it is recommended to pre-wet the pipette tip to ensure an accurate transfer of water.
10. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label. For reconstituted lyophilized reagents, follow storage requirements in section 8. Reagents Provided.
11. A calibrator curve must be established for every run.
12. It is recommended to all customers to prepare their own control materials or plasma pools which should be included in every run at a high and low level for assessing the reliability of results.
13. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing, or improper reagent storage.
14. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
15. The TMB Substrate is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
16. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored plasma.
17. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
18. Samples values above the measuring range of the kit may be reported as >800 pg/ml. If further dilution and retesting is required, only the Assay Buffer may be used to dilute EDTA plasma samples. The use of any other reagent may lead to false results.
19. Avoid microbial contamination of reagents.
20. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
21. To prevent the contamination of reagents, do not pour reagents back into the original containers.
22. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
23. Consumables used with the kit that are potentially biohazardous (e.g., pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
24. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste material containing sodium azide or sodium hypochlorite.
25. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
26. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
27. If a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
28. Do not reuse the microplate wells, they are for SINGLE USE only.
29. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
30. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.

4. SAFETY CAUTIONS AND WARNINGS

4.1 Biohazards

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

The calibrator stock, controls and assay buffer provided with the kit contain processed human serum/plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2 and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

4.2 Chemical hazards

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping Solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

5. TECHNICAL HINTS

5.1 Specimen Collection & Storage

Follow the specimen collection procedure steps in the order provided below to avoid any delays that could potentially affect the stability of specimen samples.

Approximately 0.2 ml of EDTA plasma is required per duplicate determination.

1. Prior to sample collection, place an EDTA plasma collection tube into a container of ice for at least 10 minutes.
2. Collect 4–5 ml of venous blood into an appropriately labelled pre-cooled EDTA plasma collection tube.
3. Mix the tube by inverting several times.
4. Place the collection tube into a container of ice to keep cool prior to centrifugation. Samples should not stay on the ice for more than 30 minutes.
5. Centrifuge the tube in a refrigerated centrifuge (2-8°C) at 2000x g for 10 minutes and carefully transfer the plasma into a new storage tube or container.
6. If the sample will not be tested immediately, the plasma should be aliquoted and stored frozen at ≤ -20°C for up to 3 months. Repeated freezing and thawing should be avoided.

Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

5.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

6. REAGENT SUPPLIED

6.1 Microplate

Contents: One anti-VIP polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format: Ready to Use
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.

6.2 Biotin Conjugate Concentrate Lyophilized

Contents: One bottle containing lyophilized VIP-Biotin conjugate in a protein-based buffer with a non-mercury preservative.
Format: Lyophilized and Concentrated; Requires Preparation
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label.

After Opening and Reconstitution:
Stable for 1 month at 2-8°C.
For long-term storage, store at ≤ -20°C for up to 3 months with no more than 3 freeze/thaw cycles.

Following Preparation: The Biotin Conjugate Working Solution is stable for 2 hours at room temperature following preparation. Discard the working solution after use; do not store for future use.

Reconstitution: Reconstitute the lyophilized Biotin Conjugate by adding 0.5 ml of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 10 minutes. Mix gently without foaming before use.

Preparation of Biotin Conjugate Working Solution: Dilute 1:101 Before Use
Dilute the reconstituted Biotin Conjugate 1:101 in Assay Buffer (e.g., 40 µL of reconstituted Biotin Conjugate in 4 ml of Assay Buffer) and vortex to mix.
If the whole plate is to be used dilute 80 µL of reconstituted Biotin Conjugate in 8 ml of Assay Buffer and vortex to mix.
Return unused reconstituted Biotin Conjugate back to 2-8°C or ≤ -20°C storage conditions (see stability section above).

6.3 Streptavidin HRP Conjugate

Contents: One bottle containing Streptavidin-Horse Radish Peroxidase (HRP) conjugate in a stabilized buffer with a non-mercury preservative.
Format: Ready to Use
Volume: 20 ml/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.

6.4 Calibrator Stock Lyophilized

Contents: One bottle of calibrator stock containing VIP in a human plasma-based buffer with a non-mercury preservative. Used for the preparation of VIP calibrators.
Format: Lyophilized and Concentrated; Requires Preparation
Storage: 2–8°C (unopened)
Stability: Unopened: Stable until the expiry date printed on the label.

After Opening and Reconstitution:
Store remaining reconstituted Calibrator Stock at ≤ -20°C for up to 1 month with no more than 2 freeze/thaw cycles.

Reconstitution: Reconstitute the lyophilized Calibrator Stock by adding 0.5 ml of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming before use.

Only reconstitute the Calibrator Stock immediately prior to the preparation of VIP Calibrators

Preparation of Calibrators: See section Preparation of VIP Calibrators

6.5 Control 1 – 2 Lyophilized

Contents: Two bottles of lyophilized control containing different VIP concentrations. Human plasma-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of VIP.
Refer to the QC certificate for the target values and acceptable ranges.
Format: Lyophilized; Requires Preparation
Storage: 2–8°C (unopened)
Stability: Unopened: Stable until the expiry date printed on the label.
After Opening and Reconstitution:
Stable for 2 hours at room temperature.

For long-term storage, store at ≤ -20°C for up to 1 month with no more than 2 freeze/thaw cycles.

Reconstitution: Reconstitute each bottle of control (Control 1 & Control 2) by adding 0.5 ml of distilled or deionized water to the bottle. Replace the stopper and let stand at room temperature for 2 minutes. Mix gently without foaming.

6.6 Incubation Buffer

Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
Format: Ready to Use
Volume: 6 ml/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.

6.7 Assay Buffer

Contents: One bottle containing a human plasma-based buffer with a non-mercury preservative. Used for the preparation of the VIP calibrators and the Biotin Conjugate Working Solution.
Format: Ready to Use
Volume: 20 ml/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.

6.8 TMB Substrate

Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format: Ready to Use
Volume: 18 ml/bottle

Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.

6.9 Stopping Solution

Contents: One bottle containing 1M sulfuric acid.
Format: Ready to Use
Volume: 8 ml/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks
Safety: Refer to product SDS.

6.10 Wash Buffer Concentrate

Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format: Concentrated; Requires Preparation
Volume: 50 ml/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for six weeks.
Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use.

Preparation of Wash Buffer Working Solution: Dilute 1:10 Before Use
Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of distilled or deionized water.

7. PREPARATION OF VIP CALIBRATORS

7.1 Materials Required:

– Calibrator Stock.
– Assay Buffer.
– Calibrated single-channel pipettes.
– 7 x polypropylene or HDPE tubes. (e.g. polypropylene microcentrifuge tubes).

Do not use glass test tubes; VIP may bind to glass which can alter the results.

7.2 Procedure:

An accurate preparation of the calibrators is essential to the performance of the test. Please follow good pipetting practices specific to the brand of pipettes being used

1. Label 7 x polypropylene or HDPE tubes as A, B, C, D, E, F & G, representing calibrators A-G.
2. Pipette 960 µl of Assay Buffer to tube G.
3. Pipette 500 µl of Assay Buffer to each tube A - F.
4. Reconstitute the Calibrator Stock as stated in section *8. Reagents Provided, 4. Calibrator Stock Lyophilized*.
5. Pipette 40 µl of reconstituted Calibrator Stock to tube G.
   Vortex the tube to mix thoroughly.
6. Immediately store the reconstituted Calibrator Stock at ≤ -20°C for future use.
7. Pipette 500 µl from tube G into tube F.
   Vortex tube F to mix thoroughly.
8. Pipette 500 µl from tube F into tube E.
   Vortex tube E to mix thoroughly.
9. Pipette 500 µl from tube E into tube D.
   Vortex tube D to mix thoroughly.
10. Pipette 500 µl from tube D into tube C.
    Vortex tube C to mix thoroughly.
11. Pipette 500 µl from tube C into tube B.
    Vortex tube B to mix thoroughly.

Use the prepared VIP calibrators within 2 hours after preparation. Discard any leftover calibrators; do not store for future use.

7.3 Preparation Summary Table

Calibrator	VIP (pg/ml)	Assay Buffer	Calibrator
Calibrator StockReconstituted	-	-	-
G	800	960 µl	40 µl of Calibrator Stock
F	400	500 µl	500 µl of G
E	200	500 µl	500 µl of F
D	100	500 µl	500 µl of E
C	50	500 µl	500 µl of D
B	25	500 µl	500 µl of C
A	0	500 µl	-

8. MATERIAL REQUIRED BUT NOT SUPPLIED

1. Calibrated single-channel pipette to dispense 40 – 100 µl and 500 – 1000 µl.
2. Calibrated multi-channel pipettes to dispense 25 µl, 50 µl and 150 µl.
3. Calibrated multi-channel pipettes to dispense 350 µl (if washing manually).
4. Automatic microplate washer (recommended).
5. Microplate shaker: Orbital shaker (3 mm shaking diameter) set to 600 rpm.
6. Disposable pipette tips.
7. Distilled or deionized water.
8. Calibrated absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.
9. Polypropylene or HDPE tubes for calibrator preparation (e.g. polypropylene microcentrifuge tubes).
10. Refrigerated centrifuge (2-8°C) capable of 2000x g.
11. Container with ice.
12. Vortex mixer.

9. RECOMMENDED ASSAY-LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	E	Control 2	S4	*	*	*	*	*	*	*	*
B	A	E	Control 2	S4	*	*	*	*	*	*	*	*
C	B	F	S1	*	*	*	*	*	*	*	*	*
D	B	F	S1	*	*	*	*	*	*	*	*	*
E	C	G	S2	*	*	*	*	*	*	*	*	*
F	C	G	S2	*	*	*	*	*	*	*	*	*
G	D	Control 1	S3	*	*	*	*	*	*	*	*	*
H	D	Control 1	S3	*	*	*	*	*	*	*	*	*

A-B … Calibrators

*… Samples

10. ASSAY PROCEDURE

Follow the assay procedure steps in the order provided below to avoid any delays that could potentially affect the stability of components and specimen samples.

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls, and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.

2. **Plan** the microplate wells to be used for calibrators, controls, and samples. See section Recommended Assay Layout.
   Remove the strips from the microplate frame that will not be used and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.

3. **Prepare** the Biotin Conjugate Working Solution and Wash Buffer Working Solution (See section Reagents Supplied, 2. Biotin Conjugate Concentrate Lyophilized and 10. Wash Buffer Concentrate).

4. Prepare the VIP Calibrators and Controls (See section Preparation of VIP Calibrators and section 8. Reagents Provided, 4. Calibrator Stock Lyophilized, 5. Control 1-2 Lyophilized).

5. **Pipette 25 µl** of the Incubation Buffer into each well (the use of a multi-channel pipette is recommended).

6. **Pipette 75 µL** of each calibrator, control, and specimen sample into assigned wells.

7. **Incubate** the microplate on a microplate shaker** for 30 minutes at room temperature.

8. **Pipette 50 µl** of the Biotin Conjugate Working Solution into each well (the use of a multi-channel pipette is recommended). Avoid touching the liquid in the wells with the pipette tips.

9. **Incubate** the microplate on a microplate shaker** for 60 minutes at room temperature.

10. **Wash** the microplate wells with an automatic microplate washer (preferred) or manually as stated below.

    Automatic: Using an automatic microplate washer, perform a 3-cycle wash using 350 µl/well of Wash Buffer Working Solution (3 x 350 µl). One cycle consists of aspirating all wells then filling each well with 350 µl of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.
    Manually: Perform a 3-cycle wash using 350 µl/well of Wash Buffer Working Solution (3 x 350 µl). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waste container, then pipetting 350 µl of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

11. **Pipette 150 µl** of the Streptavidin HRP Conjugate into each well (the use of a multi-channel pipette is recommended).

12. **Incubate** the microplate on a microplate shaker** for 30 minutes at room temperature.

13. **Wash** the microplate wells again as stated in step 10.

14. **Pipette 150 µl** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).

15. **Incubate** the microplate on a microplate shaker** for 30 minutes at room temperature.

16. **Pipette 50 µl** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.

17. **Measure** the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the Stopping Solution.

** See section . Material required but not supplied for microplate shaker options.

11. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 800 pg/ml and needs to be diluted and retested, then dilute with Assay Buffer not more than 1:5. The result obtained must be multiplied by the dilution factor.

12. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC Certificate.% Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

13. TYPICAL DATA

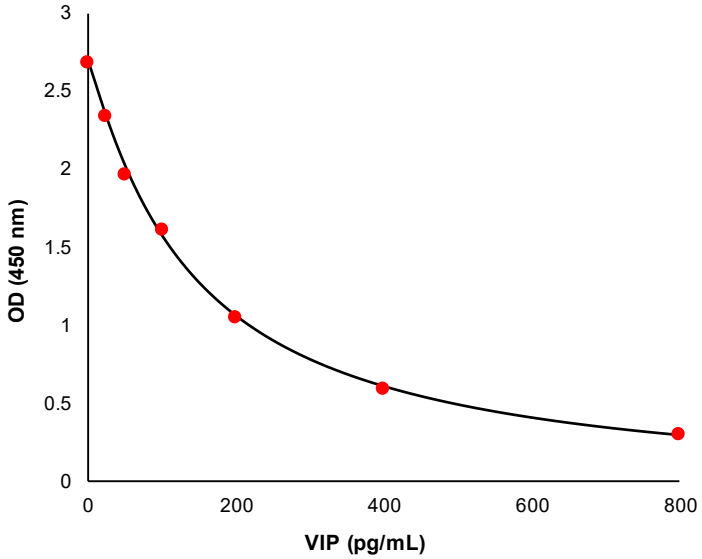
13.1 TYPICAL TABULATED DATA

Sample data only. **Do not** use to calculate results.

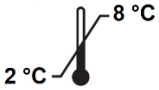
Calibrator	Mean OD (450 nm)	% Binding	Value (pg/ml)
A	2.684	100	0
B	2.346	87	25
C	1.969	73	50
D	1.613	60	100
E	1.055	39	200
F	0.595	22	400
G	0.305	11	800
Unknown	1.894	-	62.0

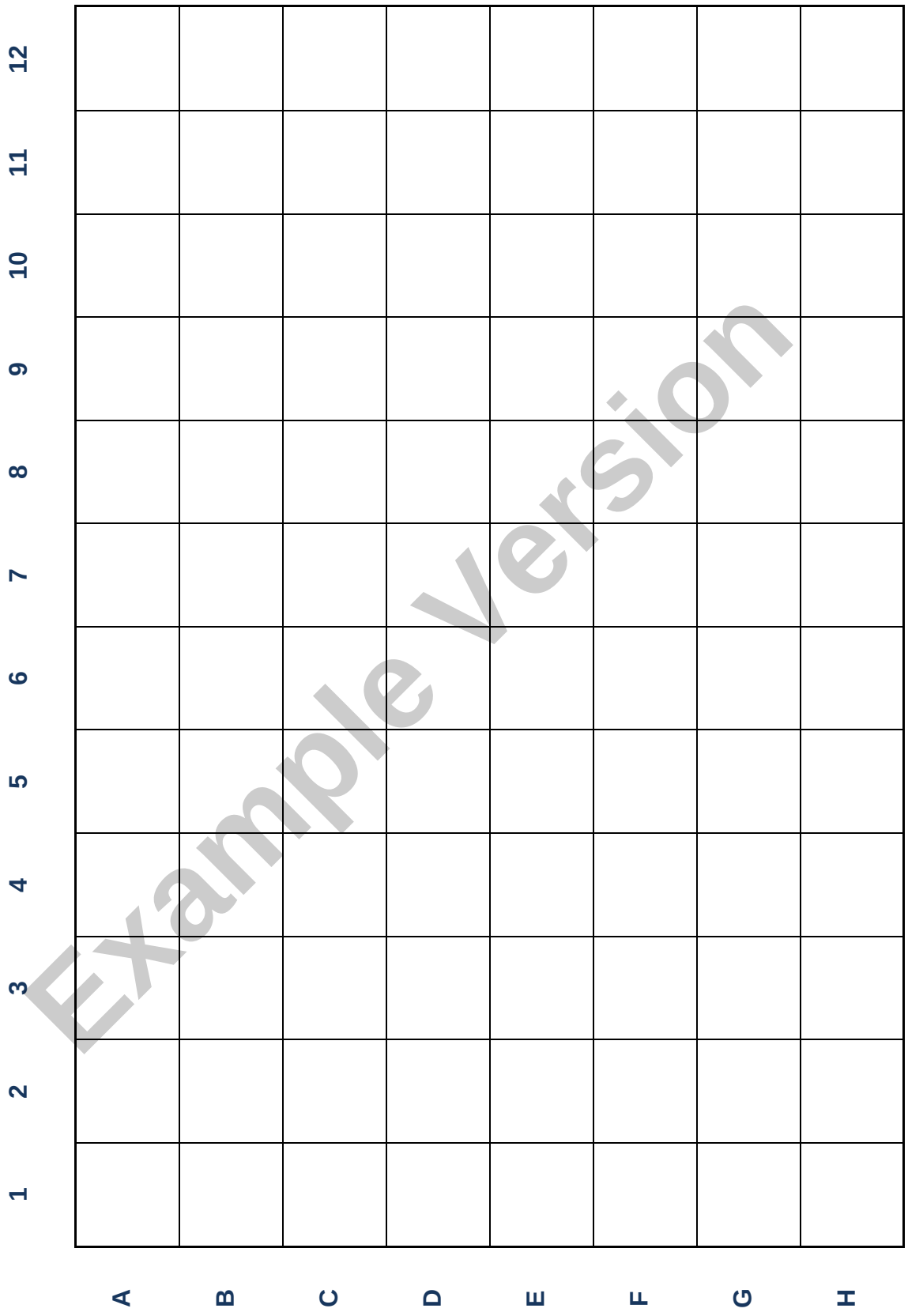
13.2 TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



14. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



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