BioVendor Group®

ENG

Instructions for Use:

RECOMBINANT FACTOR C ENDOTOXIN DETECTION KIT

Catalogue number:

RZA001R

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

Recombinant factor C endotoxin detection kit can replace the traditional limulus amebocyte lysate (LAL) for human and animal injection drugs (such as chemicals, radiopharmaceuticals, antibiotics, biological products, etc.) and medical devices (such as dialysate, implantable devices, etc.) raw and auxiliary materials, intermediate products, released products endotoxin detection. This kit is a highly sensitive, highly specific, stable and sustainably available alternative to limulus amebocyte lysate that does not depend on animal-derived components. It can measure endotoxin concentrations in the range of 0.005-5 EU /mL.

2. STORAGE AND TRANSPORTATION

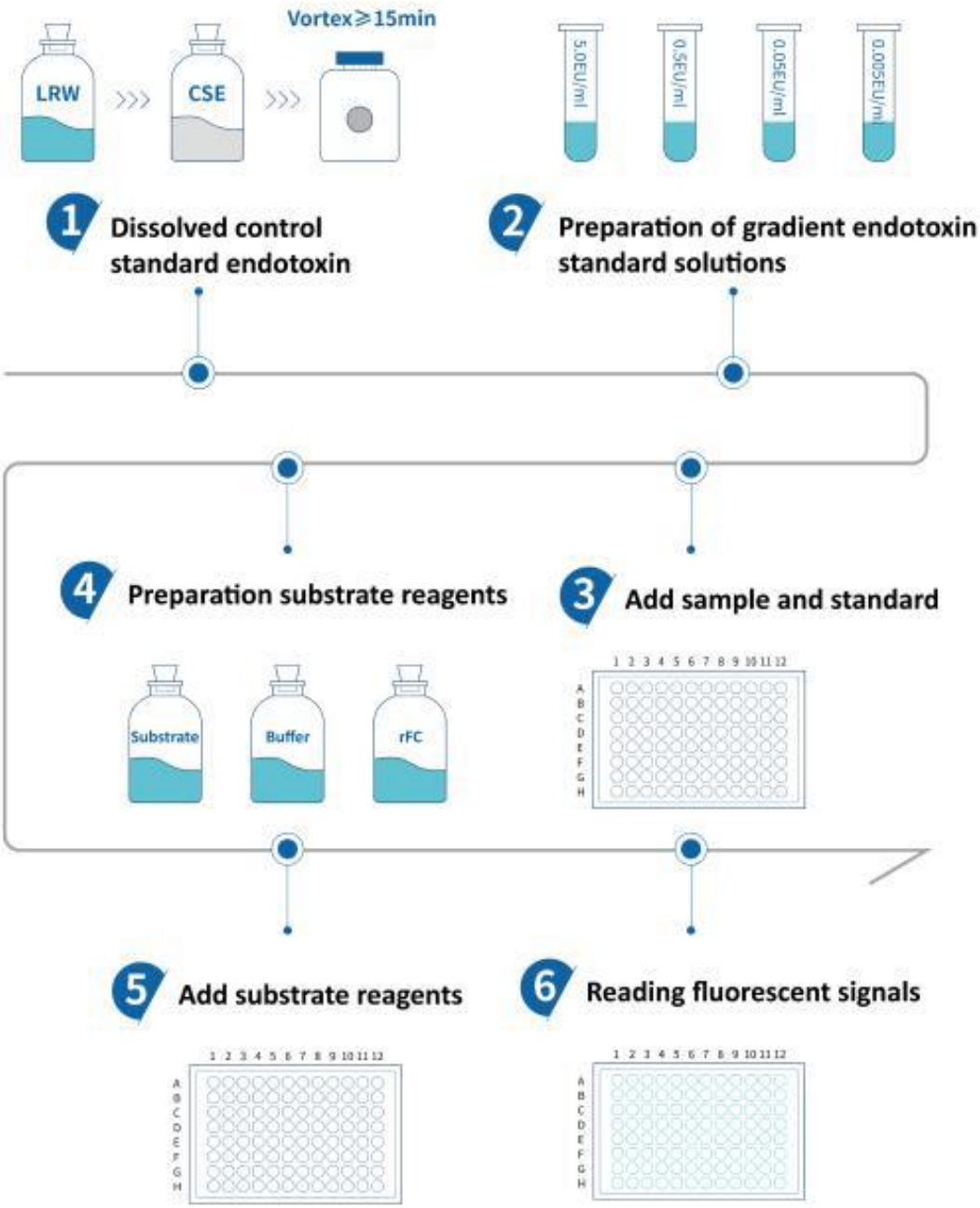
- Transport 2-8°C
- Upon receipt of the kit, store it at 2~8°C immediately
- Unopened kits are stable until the expiration date
- The control standard endotoxin should be stored at 2–8°C after reconstitution and is stable for up to 8 weeks

3. DETECTION PRINCIPLE

Limulus factor C is a serine proteinogen sensitive to bacterial endotoxin in limulus blood cells. Limulus factor C is the first to be activated by endotoxin in bacterial endotoxin-mediated limulus blood agglutination system to initiate the hemagglutination cascade in limulus blood cells. Recombinant Factor C is a recombinant factor C (rFC) expressed in the form of gene recombination. The recombinant factor C, which is bound and activated by endotoxin, can cut the substrate to obtain free fluorophores. The release of fluorophores is proportional to the concentration of endotoxin, so that endotoxin can be quantitatively detected. Compared with the classical Limulus Amebocyte Lysate (LAL) endotoxin detection method, the recombinant Factor C (rFC) assay offers higher specificity, improved precision, greater accuracy, a broader linear range, and a lower limit of quantitation. It represents an advanced alternative to the traditional LAL method for endotoxin detection.

4. TEST PRINCIPLE

The assay is performed in a 96-well microplate. Fluorescence is measured at excitation and emission wavelengths of 380 nm and 440 nm, respectively, immediately after sample addition (time 0) and again after 1 hour of incubation at 37°C ± 1°C. The net fluorescence difference is obtained by calibrating the fluorescence reading difference (ΔRFU) between the control standard endotoxin and the sample using the fluorescence difference (ΔRFU) of the negative control. The logarithm of the net fluorescence difference is proportional to the logarithm of the endotoxin concentration and is linear over the range 0.005 to 5.0EU/mL. The endotoxin concentration in the sample can be calculated according to the standard curve.



Recombinant Factor C Endotoxin Detection Kit Operation Procedure

5. PRECAUTIONS

- Recombinant factor C endotoxin detection kit is only used for in vitro endotoxin detection of samples, and cannot be used for clinical diagnosis and treatment.
- Consumables in contact with assay reagents during endotoxin detection must be clearly sterile and heat-free raw products to avoid contaminating assay reagents.
- Before use, let all reagents balance naturally to room temperature (20-25℃).
- Use a low adsorption pipette head, and use a new pipette head each time the pipette step is operated to avoid contaminating the remaining reagent.
- The use of appropriate calibrated pipettes, as far as possible to ensure the accurate transfer of all small volumes of liquids.
- Different batches of reagents shall not be mixed for use.

6. REAGENT SUPPLIED

Kit component	Quantity RZA001R
Fluorescent substrate solution	6 mL
Measuring buffer	5 mL
rFC enzyme solution	1.2 mL
LAL reagent water	30 mL
Control standard endotoxin	2 vials
Unblocking buffer 1	10 mL
Unblocking buffer 2	10 mL
96-well non-pyrogenic	1 Pc

Note: Control standard endotoxin is freeze-dried powder. See label for titer and reconstitution volume.

7. MATERIAL REQUIRED BUT NOT SUPPLIED

– Disposable pyrogen free glass dilution tube (for control standard endotoxin dilution)
– Sterile pyrogen free pipettes and suction heads
– Vortex
– Timer
– Fluorescent reader instrument capable of fluorescence detection (wavelength set to 380nm/440nm)

8. PREPARATION BEFORE EXPERIMENTS

8.1 Sample preparation

Dilute samples carefully to avoid microbial or endotoxin contamination. All materials that come into direct contact with the samples or test reagents should be pyrogen-free. Sample dilution should be performed in pyrogen-free glass dilution tubes using LAL Reagent Water. If samples cannot be analyzed immediately, they should be stored refrigerated or frozen until testing.

The pH value of samples to be tested should be maintained between 6-8. If the pH of the sample is not in this range, it needs to be adjusted with endotoxin-free sodium hydroxide, hydrochloric acid, or relevant buffer solutions.

Note: The pH of sample should not be adjusted directly using a pH electrode, as endotoxin contamination from the electrode may result in false-positive results. Preliminary studies should be performed to establish an appropriate pH adjustment procedure that minimizes the risk of endotoxin contamination.

Samples that do not interfere with (enhance or inhibit) endotoxin detection can be directly used for detection without dilution; the samples that interfere with endotoxin detection should be diluted to a concentration at which no interference is observed. During the dilution process, the sample should be thoroughly mixed by vortexing for at least 1 minute. Interference with endotoxin detection is typically caused by high concentrations of sample components. In most cases, such interference can be minimized or eliminated by appropriate sample dilution.

Prior to routine testing, sample suitability should be established to assess potential interference with the endotoxin assay. Sample interference is evaluated based on the recovery of added endotoxin. If the recovery falls outside the acceptable range, appropriate sample pretreatment, such as pH adjustment or dilution, should be applied and reassessed until acceptable recovery is achieved.

The sample dilution factor must not exceed the Maximum Valid Dilution (MVD). The MVD is calculated as follows:
MVD = Endotoxin Limit (EU/mL) ÷ Assay Sensitivity (EU/mL)
where the Endotoxin Limit is the maximum allowable endotoxin concentration in the undiluted sample, and the Assay Sensitivity is the lowest endotoxin concentration that can be reliably detected by the assay. For this kit, the assay sensitivity is 0.005 EU/mL.

8.2 Sensitivity setting of the fluorescent reader instrument

Fluorescence intensity is typically reported as Relative Fluorescence Units (RFU). Because the measured fluorescence signal is converted into an electronic signal that can be adjusted through instrument gain or sensitivity settings, RFU is an arbitrary unit. Depending on the signal intensity, the gain/sensitivity may be increased to improve detection of weak signals or decreased to prevent saturation of strong signals.
In the recombinant Factor C (rFC) endotoxin assay, log(ΔRFU) is proportional to log(endotoxin concentration, EU/mL). If the sensitivity setting is too low, the fluorescence signal of the lowest endotoxin standard may not be detected reliably. Conversely, if the sensitivity is set too high, the fluorescence signal of the highest endotoxin standard may exceed the instrument's detection

range. Therefore, the optimal fluorescence sensitivity should be established before performing the assay.
For example, the BioTek Synergy H1 microplate reader has a fluorescence detection range of 0-99,999 RFU. To ensure that all calibration standards fall within the measurable range, the RFU value for the 0.5 EU/mL endotoxin standard should be between 1,000 and 10,000 RFU (log RFU = 3-4). The midpoint of this range corresponds to approximately 3,000 RFU (log RFU = 3.5). Accordingly, a target net RFU value of approximately 3,000 RFU for the 0.5 EU/mL endotoxin standard is recommended when establishing instrument settings. This target provides adequate separation between the negative control and the lowest endotoxin standard, thereby supporting reliable assay performance during routine testing.

Note: Software interfaces and operating procedures may vary among fluorescence microplate readers from different manufacturers, users should therefore consult the user manual of their specific instrument for detailed operating instructions.

9. DETECTION STEPS

9.1 Dissolution of control standard endotoxin

- Add the corresponding volume of LAL reagent water to the lyophilized powder according to the product label to obtain a solution of 20EU/mL, and swirl for ≥15min.

Note:
1) Use a new pipette tip for each pipetting step to avoid contamination of the remaining reagents.
2) Control standard endotoxin stored under refrigerated conditions after reconstitution should be equilibrated to room temperature and mixed thoroughly (e.g., by gentle swirling) for at least 15 minutes before use.

9.2 Prepare control standard endotoxin solutions

- Take 4 disposable pyrogen free glass dilution tubes, mark the concentration of control standard endotoxin solution (0.005, 0.05, 0.5 and 5 EU/mL, respectively) on the tube, and a series of control standard endotoxin solutions are prepared with 20EU/mL of the control standard solution of endotoxin. It is recommended to dilute the control standard endotoxin by gradient dilution method as follows:

Final Concentration (EU/ml)	LAL Reagent Water	Volume of the corresponding control standard endotoxin solution
5.0	0.75 mL	0.25 mL 20EU/mL solution
0.5	0.9 mL	0.1 mL 5EU/mL solution
0.05	0.9 mL	0.1 mL 0.5EU/mL solution
0.005	0.9 mL	0.1 mL 0.05EU/mL solution

Note:

Because endotoxin may adsorb to plastic surfaces, plastic tubes should not be used for endotoxin dilutions.

Before each dilution step, vortex the control standard endotoxin solution at the preceding concentration at approximately 1400 rpm for at least 1 minute.

For example, when preparing the 5 EU/mL control standard endotoxin, the 20EU/mL control standard endotoxin solution should be sufficiently swirled at least 1 minute; when preparing 0.05 EU/mL control standard endotoxin solution, the 0.5 EU/mL control standard endotoxin solution should be swirled at least 1 minute.

Diluted control standard endotoxin solution should be used as soon as possible. Do not store the diluted control standard endotoxin solutions.

9.3 Assay setup and sample preparation

- Pipet 100µl of negative control (LAL reagent water = blank), control standard endotoxin solutions, and samples, preferably in duplicates or triplicates, into the corresponding wells of the 96-well sterile pyrogen free plate.
- It is recommended that **each sample should be tested both unspiked and spiked with a known amount of endotoxin.** Add 10µl of 5 EU/mL of control standard endotoxin solution to the spiked samples

Note: Each sample test should be performed in parallel with control standard endotoxin solutions to generate a new standard curve.

9.4 Prepare substrate reagent

- Substrate reagents should be prepared at the same time during the preparation of control standard endotoxin solutions. Equilibrate Fluorescent substrate solution, Measuring buffer and rFC enzyme solution at least 30min at room temperature.
- Prepare the substrate reagent by mixing the fluorescent substrate solution, measuring buffer, and rFC enzyme solution in a 5:4:1 ratio. Mix gently but thoroughly; do not vortex or swirl.

Note: Add the substrate reagents to a preparation container in the following order: fluorescent substrate solution, Measuring buffer, and rFC enzyme solution, ensuring that the rFC enzyme solution is added last.
Prepare the substrate reagent immediately before use; do not store the substrate reagent. Always prepare only the appropriate quantity of the substrate reagent.

9.5 Add substrate reagent and perform fluorescence measurements

- Set up the fluorescence reader parameters as follows: excitation wavelength 380 nm and emission wavelength 440 nm.
- Add the substrate reagent to each well at a volume of 100 µL/well using a pipette. Use a new pipette tip for each step to prevent cross-contamination.
- Immediately after addition of the substrate reagent, read the fluorescence signal at time point zero.
- Incubate the 96-well plate in the dark at 37±1°C. After 1 hour of incubation, read the fluorescence signal again.

9.6 Data analysis

- Export the fluorescence readings at the 0-hour and 1-hour time points from the fluorescence reader to a spreadsheet.
- Calculate the ΔRFU for each well as the difference between the fluorescence values measured at the 1-hour and 0-hour time points.
- Subtract the ΔRFU of the negative control (LAL Reagent Water, blank) from the ΔRFU of each standard and sample to obtain the net ΔRFU values.
- Construct a standard curve by plotting the net ΔRFU values of standard endotoxin solutions, or the mean net ΔRFU values when standards are measured in duplicate, against the corresponding endotoxin concentrations (EU/mL) on a logarithmic scale using a four-parameter logistic (4PL) regression model. The standard curve is considered valid if the correlation coefficient (R^2) is ≥ 0.980 and the slope is between 0.8 and 1.1.
- Determine endotoxin concentrations in samples and spiked samples. Calculate spike recovery using the following formula:

  Recovery (%) = [(spiked sample concentration − unspiked sample concentration) / 0.5 EU/mL] × 100

  The assay is considered free from interference if the recovery is within 50%–200%.
  For samples that meet the acceptance criteria, calculate the endotoxin concentration in the original (undiluted) sample by applying the appropriate dilution factor.
- Results are reported as concentration of endotoxin EU/mL in samples.

10. MEASURING RANGE AND LIMIT OF DETECTION

Measuring range: 0.005-5 EU/mL

Limit of detection: 0.005 EU/mL

11. COMMON PROBLEMS AND SOLUTIONS

11.1 Possible causes of no signal in sample detection and corresponding solutions:

- Pipetting error: Repeat the assay using calibrated pipettes and proper pipetting technique.
- Interfering substances in the sample: If the pre-test or spike recovery results are unacceptable, interference may be present. First, dilute the sample with pyrogen-free water and repeat the test. If dilution does not resolve the issue, dilute the sample using Unblocking Buffer 1 or Unblocking Buffer 2 provided with the kit to improve spike recovery. However, if dilution with pyrogen-free water is effective, the use of the unblocking buffer is not recommended.
- Sample pH is outside the acceptable range: Measure the sample pH and adjust it to the neutral range (pH 6.0–8.0) before testing.

11.2 Possible causes of low detection signal strength and corresponding solutions:

- Instrument sensitivity is too low: Increase the instrument sensitivity by adjusting the gain setting.
- Incorrect fluorescent reader parameter settings: Verify that the fluorescent reader parameters are configured correctly.
- Incubation temperature is too high or too low: Check and calibrate the incubation temperature.
- Reagent failure due to improper transportation, storage, or expiration: Verify the storage conditions, inspect the packaging for damage, and ensure the reagents have not expired.

11.3 Possible causes of high detection background values in standard and negative control Wells and corresponding solutions:

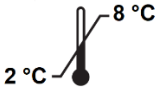
- Contamination of assay components (substrate reagent, assay buffer or rFC enzyme solution): Use newly opened reagents, and ensure that only sterile, pyrogen-free pipette tips are used.

11.4 Possible causes of poor linearity of standard curve and corresponding solutions:

- Incorrect dilution of the Control Standard Endotoxin (CSE): Prepare a new dilution of the Control Standard Endotoxin using the prescribed dilution procedure.
- The fluorescence signal from the 5 EU/ml standard exceeds the fluorescence meter's measurement range due to the instrument's insufficient sensitivity: Use a fluorescence meter with an appropriate measurement range and a wider measurement range

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data should sent to info@biovendor.com.

12. EXPLANATION OF SYMBOLS

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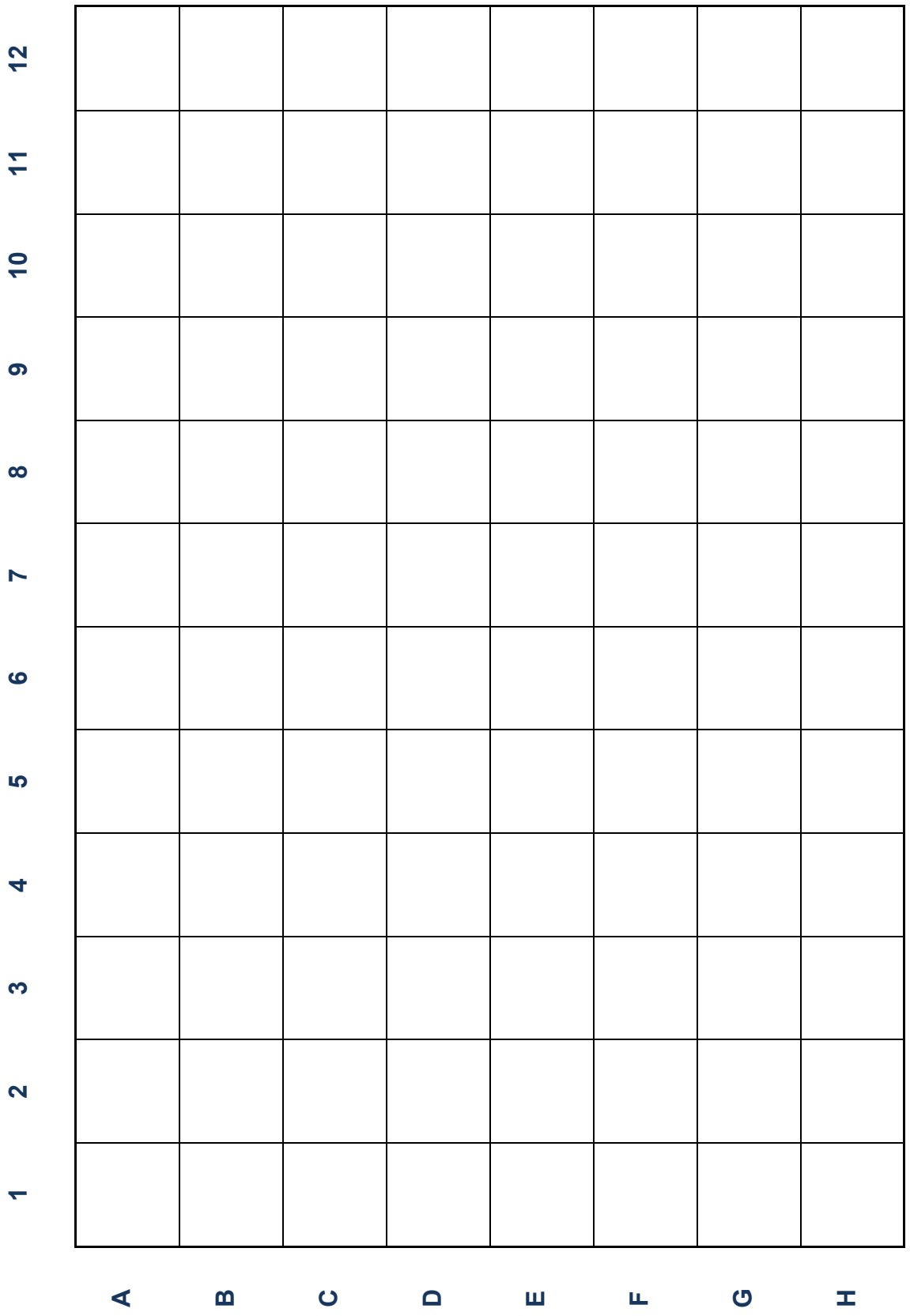
 www.biovendor.com	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks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