SHENTEK

PyroSup^{TM} MAT Kit

User Guide

PLEASE READ THE DOCUMENT CAREFULLY BEFORE EXPERIMENT

Version: A/0
Product No.: 1502100
For Research Use Only

Huzhou Shenke Biotechnology Co., Ltd.

■ Principle of the MAT

The Monocyte Activation Test (MAT) is an in vitro assay designed to detect pyrogenic substances in pharmaceutical, biological products and medical apparatus by measuring the immune response of human monocytes.

Monocytes, as key components of the human innate immune system, recognize pyrogens through Toll-like receptors (TLRs) and respond by releasing proinflammatory cytokines, such as interleukin-6 (IL-6). The concentration of IL-6 secreted into the culture supernatant reflects the degree of pyrogenic stimulation.

The PyroSup^{TM} MAT Kit utilizes cryopreserved human monocytic cells (MAT cells) and a human IL-6 ELISA assay to quantitatively determine the presence of pyrogens in test samples. This method enables detection of both endotoxin and non-endotoxin pyrogens (NEPs), providing a physiologically relevant alternative to traditional rabbit pyrogen test (PRT).

Prior to routine testing, the assay should be validated for each sample to exclude potential interference and confirm assay sensitivity for both endotoxin and non-endotoxin pyrogens.

■ Component and Material provided

Table 1. Kit Components

<table>
<tr><th>No.</th><th>Reagent</th><th>Part No.</th><th>Quantity</th><th>Storage</th></tr>
<tr><td rowspan="11">I</td><td>Culture medium</td><td>PNS003</td><td>2 × 25 mL</td><td>2-8°C</td></tr>
<tr><td>96-well plate</td><td>PNK003</td><td>1 plate</td><td>2-8°C</td></tr>
<tr><td>Water for BET</td><td>NND072</td><td>1 × 8 mL</td><td>2-8°C</td></tr>
<tr><td>Anti-hIL-6: Biotinylated Conjugate (200×)</td><td>PNG010</td><td>1 × 60 µL</td><td>2-8°C,
protect from light</td></tr>
<tr><td>Streptavidin-HRP (100×)</td><td>PNH002</td><td>1 × 140 µL</td><td>2-8°C,
protect from light</td></tr>
<tr><td>Diluent</td><td>PNE004</td><td>1 × 25 mL</td><td>2-8°C</td></tr>
<tr><td>Wash Buffer Concentrate (10×)</td><td>PNF001</td><td>2 × 25 mL</td><td>2-8°C</td></tr>
<tr><td>TMB Substrate</td><td>PND004</td><td>1 × 12 mL</td><td>2-8°C,
protect from light</td></tr>
<tr><td>Stop Solution</td><td>PNI002</td><td>1 × 6 mL</td><td>2-8°C</td></tr>
<tr><td>Sealing Film</td><td>PNK001</td><td>3 pieces</td><td>2-8°C</td></tr>
<tr><td>Anti-hIL-6 Microtiter Strips</td><td>PNA019</td><td>8 well × 12 strips</td><td>2-8°C,
protect from light</td></tr>
<tr><td rowspan="2">II</td><td>Culture medium supplement</td><td>PNR005</td><td>1 × 1.5 mL</td><td>-20°C</td></tr>
<tr><td>Reference Standard Endotoxin (RSE)</td><td>PNB019</td><td>1 vial</td><td>-20°C</td></tr>
<tr><td>III</td><td>MAT cells</td><td>PNR013</td><td>2 vials</td><td>Liquid nitrogen</td></tr>
</table>

Note:

1) *The Culture medium (PNS003), Water for BET (NND072) and Diluent (PNE004) can be purchased separately from us.*
2) *If the solution of the preparation to be examined is need tested for interference in the detection system (e.g. ELISA), the Human IL-6 ELISA Kit (Product No.: 1402431) can be purchased from us.*

Packaging

96 tests/Kit

Storage Information

All kit components can be stored under the specified conditions for up to 12 months from the date of manufacture.

Always check the expiration date indicated on each component label before use to ensure product validity.

Required but Not Provided

- Sterile, pyrogen-free pipette tips: 10 μL, 100 μL, 200 μL and 1000 μL
- Sterile, pyrogen-free centrifuge tubes with caps: 15 mL and 50 mL
- Sterile, pyrogen-free transfer pipettes: 5 mL and 10 mL
- Sterile, pyrogen-free glass tubes
- Sterile, pyrogen-free reservoirs: 50 mL
- Ultra-pure water for 1×Wash Buffer preparation

Equipment

- Desktop low-speed centrifuge (for 96-well plates)
- Water bath
- CO_2 incubator
- Biological safety cabinet (or ultra clean workbench)
- Large volume electronic dispenser
- Multi-channel micropipettes
- Vortex mixer
- Microplate shaker
- Automated microplate washer (optional)
- Cryo-freezer (-150 °C, for cell storage)
- Microplate reader with an absorbance wavelength of 450 nm and 595 nm/600 nm/620 nm

Workflow

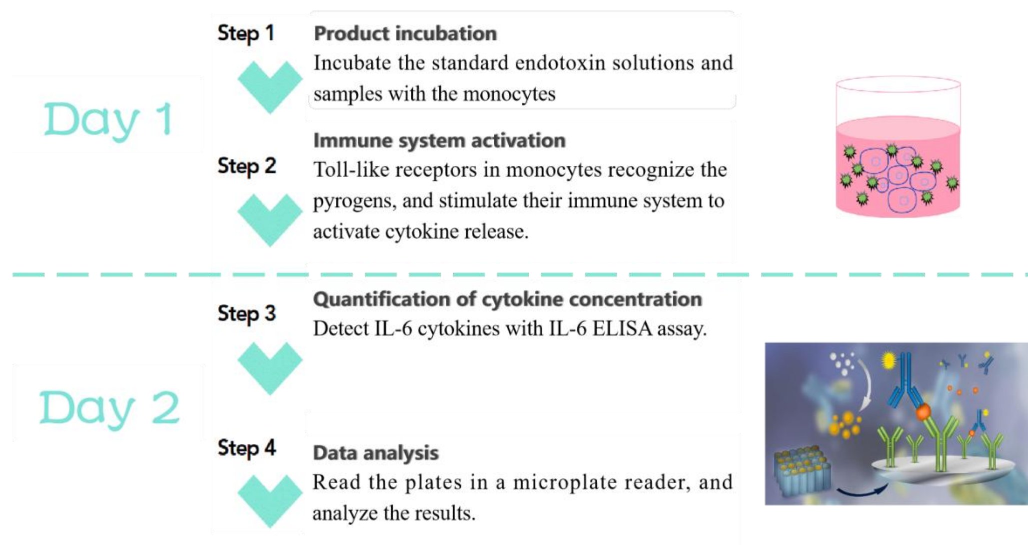


Figure 1. Procedure Flowchart

1. Day 1: Stimulation of MAT Cells

(1) Preparation of RSE Solutions

- Preparation of 30 EU/mL RSE solution: Dilute Reference Standard Endotoxin (RSE, PNB019) with suitable volume of Water for BET (NND072) according to concentration labeled on the bottle, vortex for 15 minutes to mix well, and further dilute with Water for BET to a final concentration of 30 EU/mL.

  Note:

  1) *RSE solution (30 EU/mL) can be dispensed and stored at -20℃ for 1 month.*
  2) *During the experiment, attention should be paid to zoning. The area for preparing high-concentration working standards needs to be separated from other working areas (Including samples to be tested that may contain high concentrations of pyrogens).*

- Prepare the standard curve solutions as shown in Table 2.

  Note:

  1) *The volumes for preparing each concentration can be adjusted based on experimental requirements.*
  2) *Each dilution step should be vortexed for at least 1 minute. The dilution factor between adjacent concentrations should not exceed 10-fold. The prepared RSE solutions should be used up within 4 hours.*
  3) *It is recommended to select the midpoint of the standard curve or a known*

endotoxin concentration close to it, as the spike concentration for the test sample interference experiment.

Table 2. Preparation of RSE Solutions

Tubes	Dilution Procedure	Conc. (EU/mL)
ST0	100 µL RSE solution (30 EU/mL) + 900 µL Culture medium	3.0
ST1	400 µL ST0 + 800 µL Culture medium	1.0
ST2	600 µL ST1 + 400 µL Culture medium	0.6
ST3	500 µL ST2 + 500 µL Culture medium	0.3
ST4	400 µL ST3 + 800 µL Culture medium	0.1
ST5	500 µL ST4 + 500 µL Culture medium	0.05
ST6	500 µL ST5 + 500 µL Culture medium	0.025
ST7	500 µL ST6 + 500 µL Culture medium	0.0125
NCS	Culture medium	0

(2) Sample Preparation

- Method Validation

  Before performing routine MAT testing on a given sample, method validation must be conducted to rule out potential sample interference and to confirm the assay's sensitivity in detecting endotoxins and non-endotoxin pyrogens (NEPs) in the sample matrix.

- Elimination of Interference

  Interference from the test sample can often be eliminated by diluting the sample to a certain concentration, referred to as the maximum valid dilution (MVD). The MVD is the maximum allowable dilution of a sample at which the contaminant limit can be determined. The calculation of the MVD is based on the endotoxin reference standard. Greater assay sensitivity permits higher dilution of the test sample, reliably detecting pyrogens while reducing matrix interference.

Determine the MVD using the following expression:

$$\frac{CLC \times C}{Test\ sensitivity}$$

CLC = Contaminant limit concentration;

C = Concentration of test solution;

The test sensitivity of the PyroSup^{TM} MAT kit is 0.0125 EU/mL.

Note: *Since test sensitivity is an actual point on the standard curve, it is to be confirmed (> cut-off *value) in every test to confirm the MVD calculation.*

Cut–off: The cut-off value may be calculated using the following expression:

$$\bar{x} + 3s$$

$\bar{x}$ = mean of the responses obtained for the 4 blank replicates;
s = standard deviation of the responses obtained for the 4 blank replicates.

- Interference Test Design

  The preparation of test solutions for the interference study was designed in accordance with the requirements of the European Pharmacopoeia and the Chinese Pharmacopeia. The test setup is summarized in Table 3, which lists the preparation of interference test solutions.

Table 3. Preparation of the interference test solutions

No.	Solution	Replicate	Note
①	Sample, solution / A*	4	A < MVD
②	Sample, solution / 2A	4	2A < MVD
③	Sample, solution / 4A	4	4A ≤ MVD
④	Sample, solution / A spiked with a Middle concentration from the endotoxin standard curve (Spiking RSE)	4	NA
⑤	Sample, solution / 2A spiked with a Middle concentration from the endotoxin standard curve (Spiking RSE)	4	NA
⑥	Sample, solution / 4A spiked with a Middle concentration from the endotoxin standard curve (Spiking RSE)	4	NA

***Designation of Test Solution (A):** The solution of the preparation being examined designated as corresponds to the dilution level used in the interference test. This is defined as the highest concentration or lowest dilution of the sample at which the endotoxin recovery remains within 50%-200% of the nominal value.

Note: *Vortex each endotoxin standard solution for at least 1 minute before preparing the next dilution. Each condition should be tested in four replicates. The dilution factor between adjacent concentrations should not exceed 10-fold.*

(3) Addition of Endotoxin Standards and Samples

- The recommended plate layout for sample and standard loading is shown below:

***Note:** Add 100 μL per well, and perform four replicates for each sample or standard. Vortex each dilution thoroughly before proceeding to the next dilution ensure homogeneity.*

- Routine testing should be performed only after completion of a method validation study confirming that no sample interference exists and that the assay sensitivity meets the required specifications.

✧ **Method 1: semi-quantitative test**

Table 4. Recommended plate layout (method 1)

96 well plate	1	2	3	4	5	6	7	8	9	10	11	12
A	NCS				Sample 1-①				Sample 2-①			
B	ST7				Sample 1-②				Sample 2-②			
C	ST6				Sample 1-③				Sample 2-③			
D	ST5				Sample 1-④				Sample 2-④			
E	ST4				Sample 1-⑤				Sample 2-⑤			
F	ST3				Sample 1-⑥				Sample 2-⑥			
G	ST2				Sample 1-①+NEP (optional)				Sample 2-①+NEP (optional)			
H	ST1				NEP (optional)				NA			

①~⑥ are corresponding to the numbers in Table 3.

✧ **Method 2: reference lot comparison test**

Table 5. Recommended plate layout (method 2)

96 well plate	1	2	3	4	5	6	7	8	9	10	11	12
A	Positive control (standard endotoxin)				NA				NA			
B	NCS				NA				NA			
C	Sample 1-lot 1: ①				Sample 2-lot 1: ①				Sample 3-lot 1: ①			
D	Sample 1-lot 1: ②				Sample 2-lot 1: ②				Sample 3-lot 1: ②			
E	Sample 1-lot 1: ③				Sample 2-lot 1: ③				Sample 3-lot 1: ③			
F	Sample 1-ref:* ①				Sample 2-ref: ①				Sample 3-ref: ①			
G	Sample 1-ref: ②				Sample 2-ref: ②				Sample 3-ref: ②			
H	Sample 1-ref: ③				Sample 2-ref: ③				Sample 3-ref: ③			

* **Sample-ref:** the reference lot of the sample.

①~③ are corresponding to the numbers in Table 3.

(4) Preparation of Monocyte Suspension

- Pre-warm the Culture medium (PNS003) at 37°C in water bath. Allow the Culture medium supplement (PNR005) to equilibrate to room temperature before use.
- Transport MAT cells (PNR013) from liquid nitrogen tank on dry ice.

  Note: *When taking out MAT cells from liquid nitrogen, place the vials horizontally to allow liquid nitrogen to evaporate within the tube. This prevents cracking due to internal pressure buildup.*
- Quickly thaw the cryovial of MAT cells in a 37°C water bath. Once completely thawed, remove the cryovial, disinfect the outer surface with 75% ethanol, wipe dry, and perform all subsequent operations in a biosafety cabinet.

  Note: *Hold the vial with sterile forceps, gently shake during thawing, and ensure the cap remain above the water surface.*
- Add 17 mL Culture medium and 305 µL Culture medium supplement to a 50 mL sterile, endotoxin-free centrifuge tube. Gently transfer the cells from the vial to the tube. Mix the contents by gentle pipetting or briefly vortex 3 times to avoid cell clumping.
- Transfer the MAT cell suspension into a 50 mL sterile, endotoxin-free reservoir. Using a multi-channel micropipette, dispense 150 µL each well into a 96-well plate (PNK003).
- Incubate the plate at 37°C, 5% CO_2, for 24 hours.

2. Day 2: IL-6 ELISA assay

(1) Preparation

- Before use, allow the entire ELISA kit to equilibrate at room temperature (25 ± 3°C) for 20 minutes. Return all components to 2-8°C immediately after use.
- Select the required number of Anti-hIL-6 Microtiter Strips (PNA019) according to the experimental design. Return unused strips to the foil bag with desiccant and store at 2-8°C for up to 90 days.
- 1×Wash Buffer: Dilute 1 volume of 10×Wash Buffer Concentrate with 9 volumes of ultra-pure water. For example, add 25 mL Wash Buffer Concentrate

(10×) to 225 mL of ultra-pure water to prepare 250 mL of 1×Wash Buffer. Prepare fresh and mix well before use.

Note: *If the Wash Buffer Concentrate (10×) or Diluent is cloudy or contains precipitates, warm at 37℃ until clear.*

- 1×Anti-hIL-6 Biotinylated Conjugate: Dilute the 200×Anti-hIL-6: Biotinylated Conjugate (PNG010) with Diluent in a sterile centrifuge tube to prepare the 1×Anti-hIL-6:Biotinylated Conjugate. Mix gently and prepare it freshly before use.
- 1×Streptavidin-HRP: Dilute the 100×Streptavidin-HRP (PNH002) with Diluent in a sterile centrifuge tube. Mix gently and prepare it freshly before use.

Note: *Room temperature refers to 25 ± 3℃.*

(2) Sample Loading

- After 24 hours incubation (Day 1), centrifuge the 96-well MAT cell plate at 2000 × g for 10 minutes.
- Transfer 50μL of supernatant from each well to the Anti-hIL-6 Microtiter plate (PNA019) with a multi-channel micropipette.
- Seal the plate with Sealing Film (PNK001) and incubate on a microplate thermoshaker at 600 rpm for 1 hour at room temperature, protected from light.

(3) Anti-hIL-6 Biotinylated Conjugate Incubation

- Wash the plate 3 times with 300 μL 1×Wash Buffer per well, allowing 30 seconds soak each time. Try to remove as much liquid as possible from each well and avoid drying before adding the next solution.
- Add 100 μL of freshly prepared 1×Anti-hIL-6 Biotinylated Conjugate to each well.
- Seal and incubate with Sealing Film for 45 minutes at room temperature, protected from light.

(4) Streptavidin-HRP Incubation

- Wash the plate 3 times with 300 μL 1×Wash Buffer per well, allowing 30 seconds soak each time. Try to remove as much liquid as possible from each well and avoid drying before adding the next solution.

- Add 100 µL of 1×Streptavidin-HRP solution to each well.
- Seal and incubate with Sealing Film for 30 minutes at room temperature, protected from light.

(5) Substrate Incubation

- Allow TMB Substrate (PND004) to equilibrate for 20 minutes at room temperature before use.
- Wash the plate 5 times with 300 µL 1×Wash Buffer per well, soaking 30 seconds each time. Try to remove as much liquid as possible from each well and avoid drying of wells between washes.
- Add 100 µL of TMB Substrate to each well, and incubate at room temperature for 1-10 minutes, protected from light. The maximum duration should not exceed 20 minutes.

  Note:

  1) *Do not cover the plate during TMB substrate incubation.*
  2) *The reaction mixture will gradually turn blue, add Stop Solution (PNI002) when a clear gradient of blue intensity appears in the standards.*
  3) *The Stop Solution changes the color from blue to yellow and increases signal intensity approximately threefold.*
  4) *To avoid saturation, read the plate before the highest standard reaches:*
     - $OD_{600\,nm} = 1.0$, $OD_{595\,nm} = 0.9$, $OD_{620\,nm} = 1.2$.

(6) Termination and Plate Reading

- Add 50 µL of Stop Solution to each well.
- Mix for 30 seconds and read the absorbance at 450 nm within 10 minutes.

(7) Calculation and Analysis

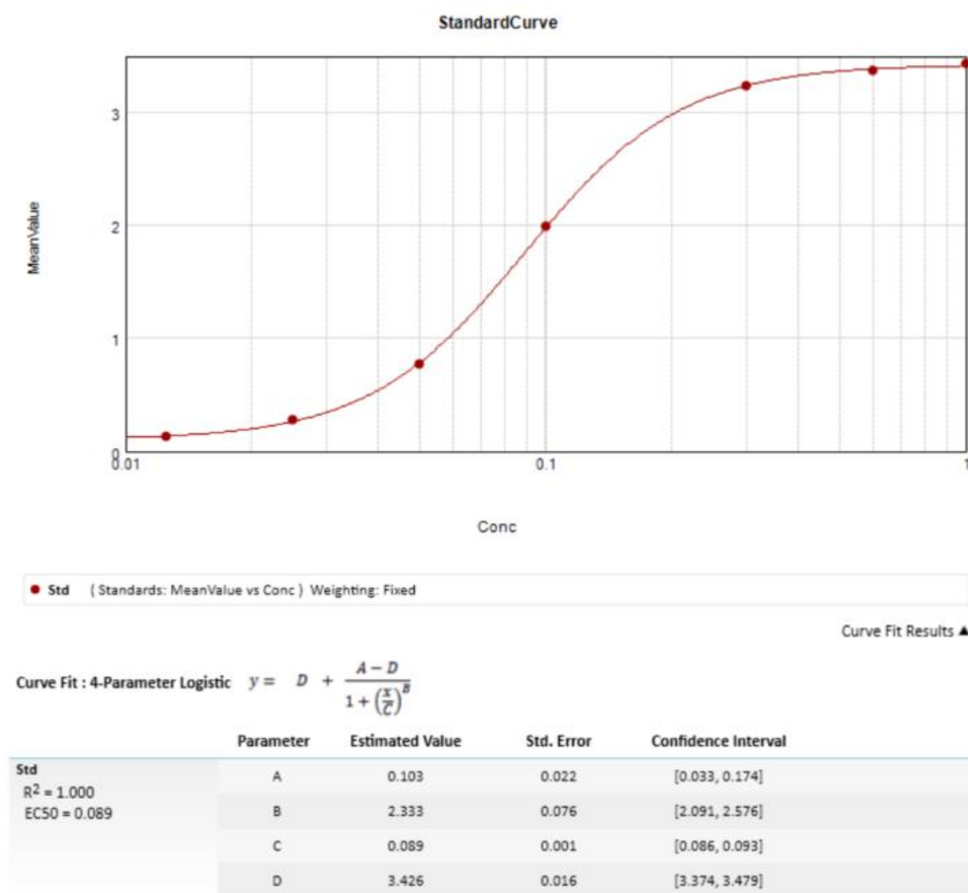
- Based on the absorbance values ($OD_{450\,nm}$) of the endotoxin standards, establish a four-parameter logistic (4-PL) standard curve.
- Calculate the endotoxin concentration of each sample from the fitted curve.
- The recovery rate can be determined as follows:

$$R = (C_{\text{with spike RSE}} - C_{\text{without spike RSE}}) / C_{\text{with spike RSE}} \times 100\%$$

- The curve must include at least five standard concentrations (points may be

removed from the extremes if necessary). The correlation coefficient R^2 should be ≥ 0.980.

- Data analysis can be performed using the microplate reader's built-in software or external tools such as CurveExpert, ELISAcalc, or equivalent.
- For samples with absorbance values above the Calibration standard ST1, determine a suitable dilution factor in a pilot study before retesting. The endotoxin concentration in test samples is calculated by multiplying the measured value by the corresponding dilution factor.
- For spiked samples tested at specific dilution levels, the recovery rate must meet the acceptance criteria defined by the relevant pharmacopoeial or regulatory guidelines.



Parameter	Estimated Value	Std. Error	Confidence Interval
A	0.103	0.022	[0.033, 0.174]
B	2.333	0.076	[2.091, 2.576]
C	0.089	0.001	[0.086, 0.093]
D	3.426	0.016	[3.374, 3.479]

Figure 2. The endotoxin standard curve (Example)

(8) Pyrogenicity of the Sample

- For each sample dilution within the range that does not exceed the MVD, the endotoxin spike recovery is calculated based on the measured endotoxin concentration (EU/mL) of the spiked and non-spiked samples.
- The spike recovery is considered valid only when it falls within the range of 50%–200%. Any dilution that does not meet this criterion is deemed invalid and must be further diluted or otherwise treated to eliminate matrix interference. If the interference cannot be eliminated, the test shall not be continued.
- For each validated sample dilution, the OD values are converted to endotoxin concentrations (EU/mL), using the standard curve, multiplied by the corresponding dilution factor, then compared with the CLC to determine whether the sample exhibits pyrogenic activity:

✧ **Method 1:**

If the value (in EU/mL) of sample is **< CLC→PASS**, indicates that the pyrogen level in the sample is below the CLC.

If the value (in EU/mL) of sample is **> CLC→FAIL**, indicates that the pyrogen level in the sample is above the CLC.

✧ **Method 2:**

The positive control and at least one dilution of the reference lot should be above the mean OD value of the blank.

For each sample, an OD ratio is calculated, corresponding to the sum of the mean response of the 3 dilutions of the lot being examined divided by the sum of the mean response of the 3 dilutions of the reference lot. The OD ratio should not exceed a justified acceptance criterion. This acceptance criterion is to be defined by the end-user.

If the OD ratio is **≤ of the** acceptance criterion→**PASS**, indicates that the lot being examined is not pyrogenic compared to the reference lot.

If the OD ratio is **> of the** acceptance criterion→**FAIL**, indicates that the lot being examined is pyrogenic compared to the reference lot.

Assay Performance

- Range of the standard curve: 0.0125-1.0 EU/mL (optional, points ≥ 5), 4-PL, $R^2 \geq 0.980$
- QL: 0.025 EU/mL
- DL: 0.0125 EU/mL

Note: Endotoxin standard curve and the cut-off value need to be calculated for each individual experiment.

Additional Information

- ✧ This kit is intended for use by qualified personnel trained in cell-based assay techniques
- ✧ Consumables (*e.g.* sterile, pyrogen-free pipette tips, tubes, and reservoirs) are for single use only. Wipe work surfaces with 75% ethanol before and after use. Follow the specified pipetting procedures carefully.
- ✧ Assay validation must be performed prior to testing routine samples.
- ✧ The operating environment, as well as all experimental materials and consumables, must be maintained sterile and pyrogen-free.
- ✧ Endotoxin standards and samples dilutions should be prepared in pyrogen-free glass tubes. The use of plastic tubes or EP tubes is not recommended for ensure not adsorbed during the dilution process with the endotoxin standards. Ensure sufficient mixing time at each dilution step to achieve complete homogenization.
- ✧ This method is not applicable to test substance which stimulate or inhibit monocyte cytokine release, or significantly affect cell proliferation.
- ✧ Perform all dilution steps gently to avoid excessive foaming which may introduce assay variability.
- ✧ Handle the Stop Solution (1M HCl) with care to avoid contact with eyes, skin, and clothing.
- ✧ It is recommended to adjust the pH of the test sample to 6.0-8.0 before cell incubation to ensure optimal assay performance.

References

- Ph. Eur. <2.6.30> Monocyte activation test
- ChP <9301> Guidelines for the Application of Safety Test Methods for Injections

Effective date: 28 Nov. 2025

Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189