



Kininogen-1/KNG1 ELISA Kit (Human)
(OKBB00653)
Lot# KH0055

Instructions for Use

For the quantitative measurement of Human Kininogen-1 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

Table of Contents

| | |
|--|----|
| 1. Background | 2 |
| 2. Assay Summary | 3 |
| 3. Storage and Stability..... | 3 |
| 4. Kit Components | 3 |
| 5. Precautions | 4 |
| 6. Required Materials Not Supplied | 4 |
| 7. Technical Application Tips | 4 |
| 8. Reagent Preparation..... | 5 |
| 9. Sample Preparation | 7 |
| 10. Assay Procedure | 8 |
| 11. Calculation of Results..... | 9 |
| 12. Typical Expected Data | 9 |
| 13. Technical Resources | 11 |

1. Background

Principle

Aviva Systems Biology Kininogen-1/KNG1 ELISA Kit (Human) (OKBB00653) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. A mouse monoclonal antibody specific for Kininogen-1 has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (NSO; Q19-S644) and test samples are added to the wells and incubated. After washing, a biotinylated polyclonal goat detector antibody specific for Kininogen-1 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Human Kininogen-1 captured in well.

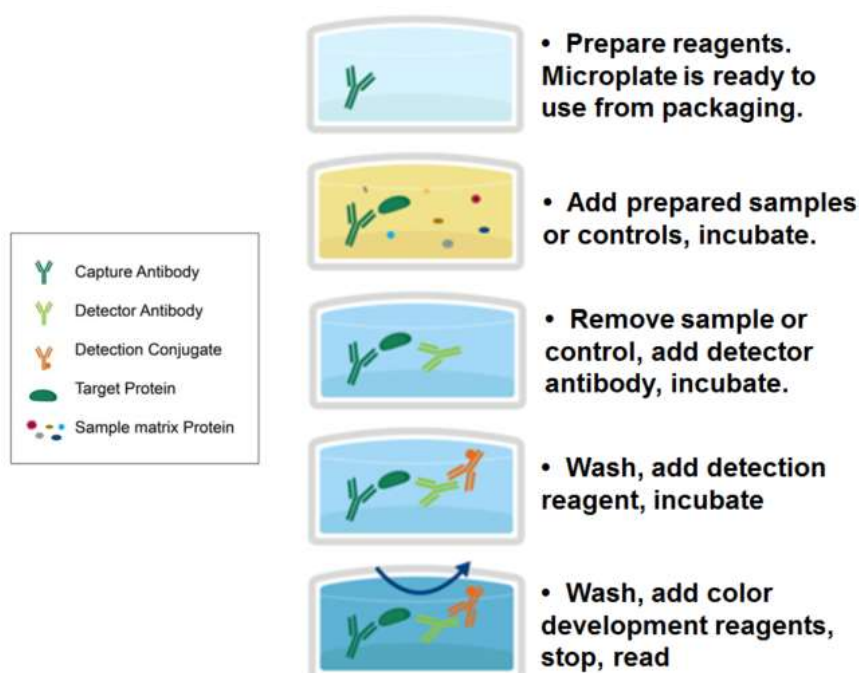
Background

Kininogens are inhibitors of thiol proteases; (2) HMW-kininogen plays an important role in blood coagulation by helping to position optimally prekallikrein and factor XI next to factor XII; (3) HMW-kininogen inhibits the thrombin- and plasmin-induced aggregation of thrombocytes; (4) the active peptide bradykinin that is released from HMW-kininogen shows a variety of physiological effects: (4A) influence in smooth muscle contraction, (4B) induction of hypotension, (4C) natriuresis and diuresis, (4D) decrease in blood glucose level, (4E) it is a mediator of inflammation and causes (4E1) increase in vascular permeability, (4E2) stimulation of nociceptors (4E3) release of other mediators of inflammation (e.g. prostaglandins), (4F) it has a cardioprotective effect (directly via bradykinin action, indirectly via endothelium-derived relaxing factor action); (5) LMW-kininogen inhibits the aggregation of thrombocytes; (6) LMW-kininogen is in contrast to HMW-kininogen not involved in blood clotting.

General Specifications

| General Specifications | |
|------------------------|---|
| Range | 0.156 – 10 ng/mL |
| Sensitivity | < 0.02 ng/mL (Derived by linear regression of OD ₄₅₀ of the Mean Blank + 2xSD) |
| Specificity | Natural and recombinant Human Kininogen-1 <u>UniProt ID:</u> P01042 <u>Gene ID:</u> 3827 <u>Target Alias:</u> Alpha-2-thiol proteinase inhibitor, BDK, Fitzgerald factor, High molecular weight kininogen, HMWK, Kininogen-1, KNG, Williams-Fitzgerald-Flaujeac factor |
| Cross-Reactivity | No detectable cross-reactivity with other relevant proteins |

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at -20°C until expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

- The following reagents are the provided contents of the kit.

| Description | Quantity | Storage Conditions |
|---|-----------------------|--------------------------------------|
| 96-Well plate Pre-coated with Anti-Human Kininogen-1 Antibody | 1 (12 x 8 Well Strip) | Store at -20°C until expiration date |
| Lyophilized Recombinant Human Kininogen-1 Standard | 2 x 50 ng | |
| 100X Biotinylated Anti-Human Kininogen-1 Antibody | 1 x 100 µL | |
| 100X Avidin-Biotin-Peroxidase Complex (ABC) | 1 x 100 µL | |
| Sample Diluent Buffer | 1 x 30 mL | |
| Antibody Diluent Buffer | 1 x 12 mL | |
| ABC Diluent Buffer | 1 x 12 mL | |
| TMB Color Developing Agent | 1 x 10 mL | |
| TMB Stop Solution | 1 x 10 mL | |
| 10X Wash Buffer | 1 x 30 mL | |

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 μ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
- Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results in inter- intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 μ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates or fibrin strands or which are hemolytic or lipemic might cause inaccurate results due to interfering factors.

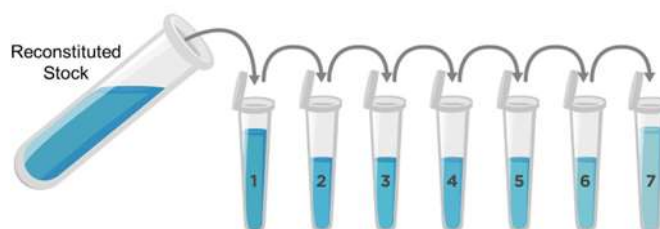
8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use immediately.

8.1 KNG1 Assay Standards

- 8.1.1 Prepare the KNG1 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.
- 8.1.2 Reconstitute one of the provided 50 ng **Lyophilized Recombinant Human KNG1 Standard**. Use one for each experiment. Prepare a stock 50 ng/mL **Human KNG1 Standard** by reconstituting one tube of **Lyophilized Recombinant Human KNG1 Standard** as follows:
 - 8.1.2.1 Gently spin or tap the vial to collect all material at the bottom.
 - 8.1.2.2 Add 1 mL of **Sample Diluent Buffer** to the vial.
 - 8.1.2.3 Seal then mix gently and thoroughly.
 - 8.1.2.4 Leave the vial to sit at ambient temperature for 10 minutes.
- 8.1.3 Prepare a set of seven serially diluted standards as follows:
 - 8.1.3.1 Label tubes with numbers 1 – 8.
 - 8.1.3.2 Add 300 µL of **Sample Diluent Buffer** to Tube #'s 2 – 8.
 - 8.1.3.3 Prepare a **10 ng/mL Standard #1** by adding 200 µL of the **50 ng/mL** reconstituted **Human KNG1 Standard** to 800 µL of **Sample Diluent Buffer** in Tube#1. Mix gently and thoroughly.
 - 8.1.3.4 Prepare **Standard #2** by adding 300 µL of **10 ng/mL Standard #1** from Tube #1 to Tube #2. Mix gently and thoroughly.
 - 8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.
 - 8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.
 - 8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent Buffer**), which should be included with every experiment.

| Standard Number (Tube) | Sample To Dilute | Volume Standard (µL) | Volume Sample Diluent (µL) | Total Volume (µL) | Final Concentration |
|------------------------|-------------------------------------|----------------------|----------------------------|-------------------|---------------------|
| Tube #1 | 50 ng/mL Human Kininogen-1 Standard | 200 | 800 | 1,000 | 10 ng/mL |
| Tube #2 | 10 ng/mL | 300 | 300 | 600 | 5.0 ng/mL |
| Tube #3 | 5.0 ng/mL | 300 | 300 | 600 | 2.5 ng/mL |
| Tube #4 | 2.5 ng/mL | 300 | 300 | 600 | 1.25 ng/mL |
| Tube #5 | 1.25 ng/mL | 300 | 300 | 600 | 0.625 ng/mL |
| Tube #6 | 0.625 ng/mL | 300 | 300 | 600 | 0.312 ng/mL |
| Tube #7 | 0.312 ng/mL | 300 | 300 | 600 | 0.156 ng/mL |
| Tube #8 | NA | 0 | 300 | 300 | 0.0 (Blank) |



8.2 1X Biotinylated Anti-Human Kininogen-1 Antibody

- 8.2.1 Prepare the **1X Biotinylated Anti-Human Kininogen-1 Antibody** immediately prior to use by diluting the **100X Biotinylated Anti-Human Kininogen-1 Antibody** 1:100 with Antibody Diluent Buffer.
- 8.2.2 For each well to be used in the experiment prepare 120 μ L by adding 1.2 μ L of **100X Biotinylated Anti-Human Kininogen-1 Antibody** to 118.8 μ L Antibody Diluent Buffer.
- 8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.3 1X Avidin-Biotin-Peroxidase Complex (ABC)

- 8.3.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC)** 1:100 with **ABC Dilution Buffer**.
- 8.3.2 For each well to be used in the experiment prepare 120 μ L, by adding 1.2 μ L of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 118.8 μ L **ABC Dilution Buffer**.
- 8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.4 1X Wash Buffer

- 8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.
- 8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.
- 8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.
- 8.4.4 Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Clear samples by centrifugation as follows:
 - **Cell culture supernatants** - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
 - **Cell Lysates** - Collect cells and rinse cells with PBS. Homogenize and lyse cells thoroughly in lysate solution (Cell Protein Extraction Reagent, Catalog# OKBB00019). Centrifuge cell lysates at approximately 10000 X g for 5 min to remove debris. Aliquots of the cell lysates were removed and assayed.
 - **Serum** - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
 - **Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

- Prepare 150 µL sample for each replicate to be assayed.
- Dilute samples with Sample Diluent Buffer.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

| Estimated Sample Target Concentration | | Dilution Level | Sample Volume For Two Replicates | Sample Diluent Buffer For Two Replicates |
|---------------------------------------|-----------------|--------------------|----------------------------------|--|
| High Concentration | 500-5,000 ng/mL | 1:100 | 3 µL | 297 µL |
| Medium Concentration | 50-500 ng/mL | 1:10 | 30 µL | 270 µL |
| Low Concentration | 0.78-50 ng/mL | 1:2 | 150 µL | 150 µL |
| Very Low Concentration | ≤0.78 ng/mL | 1:2 or No Dilution | - | - |

10. Assay Procedure

- The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.
- To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

- 10.1** Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.
- 10.2** Cover the plate with the plate sealer and incubate for 90 minutes.
- 10.3** Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- 10.4** Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.5** Add 100 µL of prepared **1X Biotinylated Anti-Human KNG1 Antibody** to each well.
- 10.6** Cover with the plate sealer and incubate for 60 minutes.
- 10.7** Wash plate 3 times with **1X Wash Buffer** as follows:
 - 10.7.1 Add 300 µL of **1X Wash Buffer** to each assay well.
 - 10.7.2 Incubate for 1 minute.
 - 10.7.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
 - 10.7.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
 - 10.7.5 Repeat steps 10.9.1 through 10.9.4 **two** more times
- 10.8** Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC)** into each well, cover with plate sealer and incubate for 30 minutes.
- 10.9** Wash plate 5 times with **1X Wash Buffer** as follows:
 - 10.9.1 Add 300 µL of **1X Wash Buffer** to each assay well.
 - 10.9.2 Incubate for 1 minute.
 - 10.9.3 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
 - 10.9.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
 - 10.9.5 Repeat steps 10.13.1 through 10.13.4 **four** more times.
- 10.10** Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate in the dark for 15-25 minutes.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)
- 10.11** Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.
- 10.12** Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.15.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD₄₅₀** for each test or standard well as follows:

$$(\text{Relative OD}_{450}) = (\text{Well OD}_{450}) - (\text{Mean Blank Well OD}_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD₄₅₀** of each standard serial dilution point vs. the respective standard concentration. The Human Kininogen-1 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD₄₅₀** against the standard curve. This is best achieved using curve fitting software.

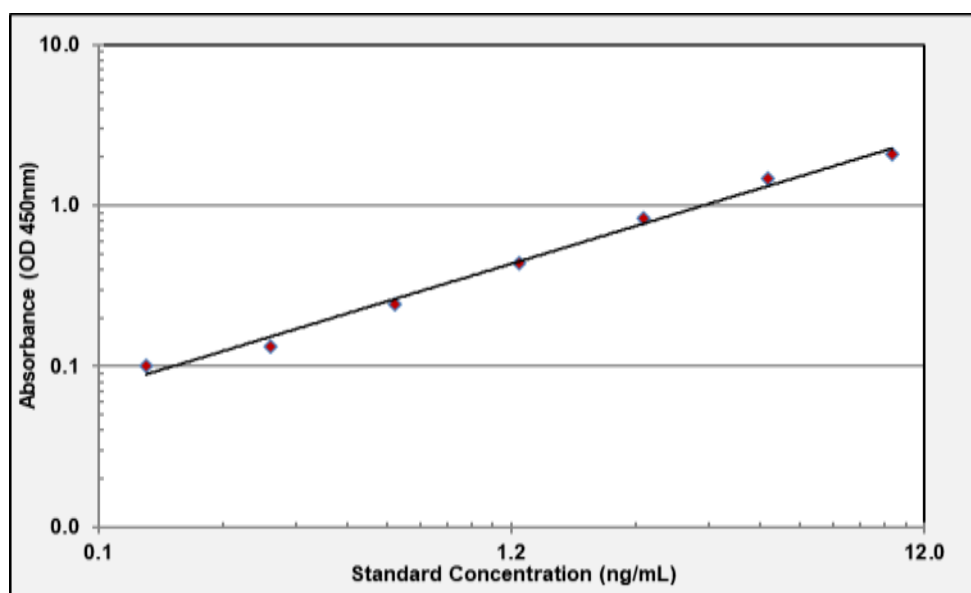
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD₄₅₀.

| Standard Number | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Concentration (ng/mL) | 0 | 0.156 | 0.312 | 0.625 | 1.25 | 2.5 | 5.0 | 10 |
| OD ₄₅₀ | 0.051 | 0.101 | 0.134 | 0.243 | 0.439 | 0.829 | 1.482 | 2.072 |

12.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Reproducibility

| | Intra-Assay | | | Inter-Assay | | |
|-------------------------------------|-------------|-------|--------|-------------|-------|--------|
| Sample ID | 1 | 2 | 3 | 1 | 2 | 3 |
| n = | 16 | 16 | 16 | 24 | 24 | 24 |
| Mean Measured Concentration (pg/mL) | 221 | 658 | 3779 | 249 | 672 | 4128 |
| Standard Deviation | 10.83 | 33.56 | 219.18 | 17.68 | 51.07 | 321.98 |
| Consistency (%CV) | 4.9 | 5.1 | 5.8 | 7.1 | 7.6 | 7.8 |

13. Technical Resources

13.1 References

- 13.1.1 Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ (February 1989). "Structure and function of Human Kininogen-1 : a member of the epidermal growth factor family". Science 243 (4894 Pt 1): 1074–6.
- 13.1.2 Zaiss, D. M., Yang, L., Shah, P. R., Kobie, J. J., Urban, J. F., Mosmann, T. R. Kininogen-1, a T(H)2 cytokine enhancing resistance to nematodes. Science 314: 1746 only, 2006.
- 13.1.3 Ciarloni, L., Mallepell, S., Briskin, C. Kininogen-1 is an essential mediator of estrogen receptor alpha function in mammary gland development. Proc. Nat. Acad. Sci. 104: 5455-5460, 2007.

13.2 Technical Support

USA

Aviva Systems Biology, Corp.
10211 Pacific Mesa Blvd, Ste 401
San Diego, CA 92121

Phone: 858-552-6979
Toll Free: 888-880-0001
Fax: 858-552-6975

Technical support: techsupport@avivasysbio.com

China

Beijing AVIVA Systems Biology
6th Floor, B Building, Kaichi Tower
#A-2 Jinfu Road.
Daxing Industrial Development Zone
Beijing, 102600, CHINA

Phone: (86)10-60214720
Fax: (86)10-60214722
E-mail: support@avivasysbio.com.cn

中国地址: 北京大兴工业开发区金辅路甲 2 号凯驰大厦 B 座 6 层 (102600)

电话: 010-60214720/21

传真: 010-60214722

产品售前咨询及销售: sales@avivasysbio.com.cn

售后及技术支持: support@avivasysbio.com.cn