



# 從COVID-19看新型傳染病之 檢測開發、試驗與量產

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AllBio Science, Inc.  
百歐精準生物醫學股份有限公司  
AllBio Life, Inc.



# 百歐提供的服務項目

Gene Synthesis(基因合成)

Protein Expression(蛋白質表現)

Customized Antibody(客製化抗體)

Rapid Test Kits(快篩試劑)

Next Generation Sequencing(次世代定序)

Precision Medicine(精準醫學)

eHealth Care(數位健康照護)

Published OnlineFirst August 20, 2019; DOI: 10.1158/0008-5472.CAN-19-1402

Molecular Cell Biology

Cancer Research

Published OnlineFirst August 20, 2019; DOI: 10.1158/0008-5472.CAN-19-1402

## MAP4K3/GLK Promotes Lung Cancer Metastasis via Phosphorylating IQGAP1

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MAP4K3/GLK Promotes Lung Cancer Metastasis via IQGAP1

### Abstract

Overexpression of the serine/threonine kinase MAP4K3 in human lung cancer is associated with poor prognosis. However, the molecular mechanism remains unclear. Here, we report that GLK promotes tumor metastasis and cell invasion via phosphorylating IQGAP1. GLK overexpression in lung cancer cells promotes cell invasion and metastasis. IQGAP1 was identified as a protein that interacts with GLK. Co-immunoprecipitation and pull-down assays demonstrated that GLK-mediated phosphorylation of IQGAP1 is mediated by the kinase. In addition, the binding site of GLK on IQGAP1 is located at the C-terminal region. These results suggest that GLK promotes lung cancer metastasis via phosphorylating IQGAP1.

### Introduction

More than 90% of human cancer-related deaths are caused by lung cancer (1,2). Cancer cell migration and metastasis (3). Understanding the mechanisms of cancer cell migration should help develop therapeutic approaches for treating cancer. IQGAP1 is a scaffolding protein that interacts with a variety of proteins, including GTPase-activating protein (GAP), GTPase-activating protein (GAP), and GTPase-activating protein (GAP).

### IHC

Tissue sections were deparaffinized, and then treated for antigen retrieval by incubating the slides in boiling buffer (pH 6.0) at 85°C for 10 minutes. Nonspecific binding was sequentially blocked with 3% H<sub>2</sub>O<sub>2</sub> for 10 minutes and Immunoblock-Ultra V block for 5 minutes. Tissue sections were incubated with anti-proliferating cell nuclear antigen (PCNA; 1:200; GeneTex) or anti-EGFR<sup>858</sup> antibodies (1:200; Cell Signaling Technology) at 4°C overnight, and then incubated with HRP-conjugated secondary antibodies. The protein signals were detected using the DAB substrate 3,3'-diaminobenzidine (DAB; Ultravision Quanto Detection System; Thermo Fisher Scientific, TI-060-QH). For negative controls, primary antibodies were replaced with 2% normal serum. Tissue sections were also counterstained with Mayer hematoxylin.

### Time-lapse super-resolution live-cell imaging

For monitoring subcellular localization of GLK-mGFP and IQGAP1-Tomato in migrating cells, 2 × 10<sup>5</sup> cells were seeded into 8-chamber slides 24 hours after transfection. After a further 24 hours of incubation, cells were traced using Nikon Structured Illumination Microscope (N-SIM) performed on an Eclipse Ti inverted microscope equipped with a Plan Apo ×60 water immersion objective and time-lapse live-cell imaging system (Nikon). Motile transfected (mGFP- and Tomato-positive) cells were followed in time-lapse recording for 10 hours at an interval of 10 minutes. The images were acquired and analyzed with the NIS Elements software (Nikon).

Se-480 was generated by immunization of a mouse with phospho-peptides (human IQGAP1 epitope: <sup>37</sup>NTVWRQLpS SSVTLGL<sup>480</sup>). The tissue sections were then incubated with species-specific secondary antibodies conjugated with oligonucleotides (PLA probes), followed by ligation and amplification reactions. The number of PLA signals per tissue (3.14 mm<sup>2</sup>) was counted.

### Statistical analysis

All experiments were repeated at least three times. The associations between metastasis and GLK transgene were evaluated using the Fisher exact test. To evaluate normality of each column data, Kolmogorov-Smirnov and Shapiro-Wilk tests were performed. The statistical significance between two unpaired groups was analyzed using the two-tailed Student *t* test (for normally distributed data) or using the two-tailed Mann-Whitney *U* test (for nonnormally distributed data). Cluster analyses (hierarchical clustering and subsequent k-means clustering) were used to divide patients into subgroups. Kaplan-Meier survival analyses were performed to show the difference in the survival between subgroups (e.g., PLA signal-High vs. PLA signal-Low). The log-rank test was used to calculate the significance of the survival distributions between two groups. Data were calculated using SPSS 19 software. A *P* value of <0.05 was considered statistically significant (\*, *P* < 0.05; \*\*, *P* < 0.01; \*\*\*, *P* < 0.001). All statistical analyses of clinical data were further independently verified by two biostatisticians at Institute of Population Sciences of National Health Research Institutes (Zhunan, Taiwan).

For experiments using human pulmonary tissues, tissue sections were deparaffinized, antigen retrieved, and nonspecific-binding blocked, followed by *in situ* PLA assays using first antibodies for IQGAP1 (1:4,000, CUSABIO) plus either GLK (1:3,000, mAb clone C3), or phospho-IQGAP1 Ser-480 (1:2,000, Allbio Science). The mAb for phosphorylated IQGAP1

4078 Cancer Res 79(30) October 1, 2019

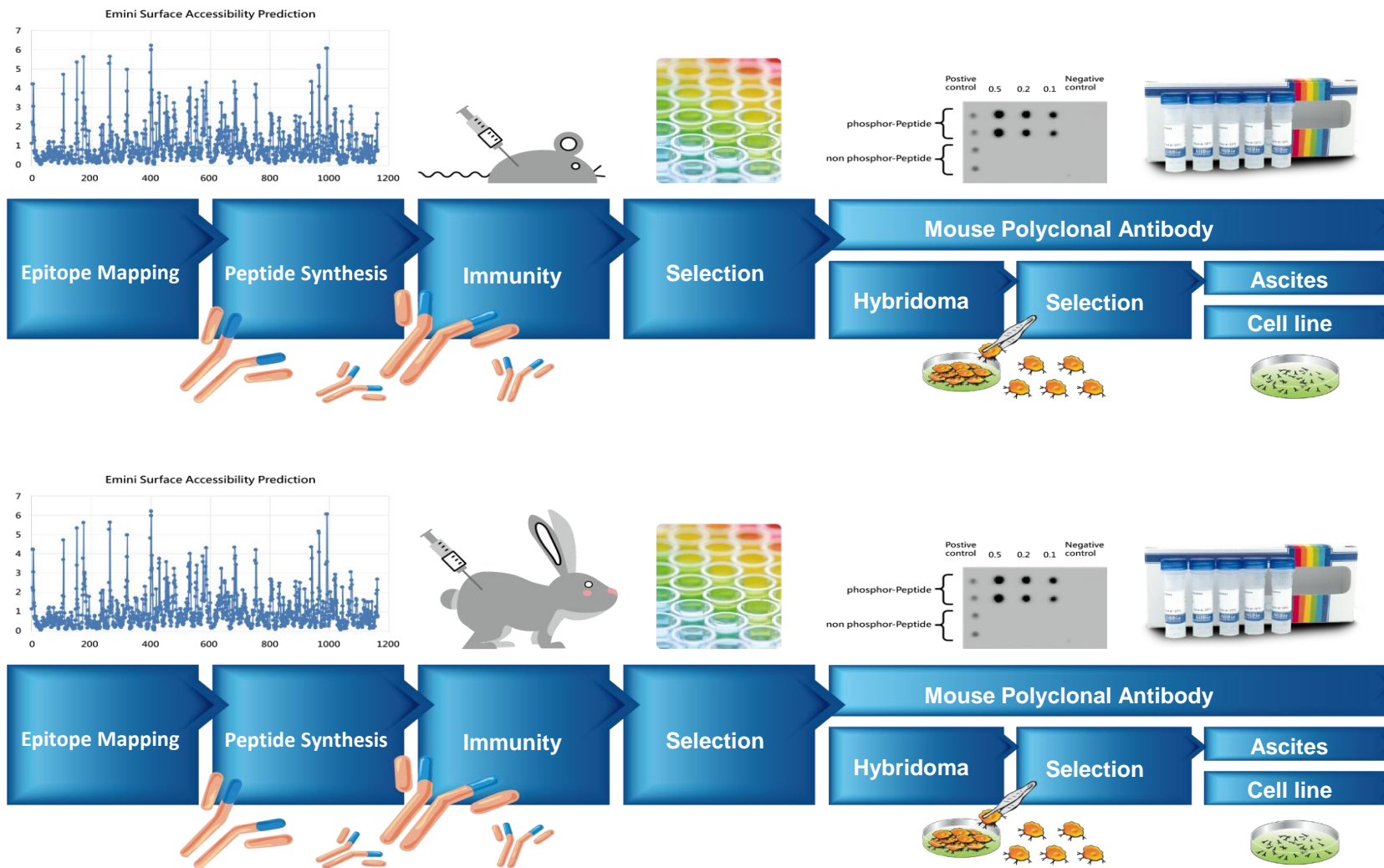
Downloaded from [cancerres.aacrjournals.org](https://cancerres.aacrjournals.org)

ments, at least five different fields were randomly selected, and the number of red spots per cell was counted. Each experiment was repeated at least three times.

For experiments using human pulmonary tissues, tissue sections were deparaffinized, antigen retrieved, and nonspecific-binding blocked, followed by *in situ* PLA assays using first antibodies for IQGAP1 (1:4,000, CUSABIO) plus either GLK (1:3,000, mAb clone C3), or phospho-IQGAP1 Ser-480 (1:2,000, Allbio Science). The mAb for phosphorylated IQGAP1

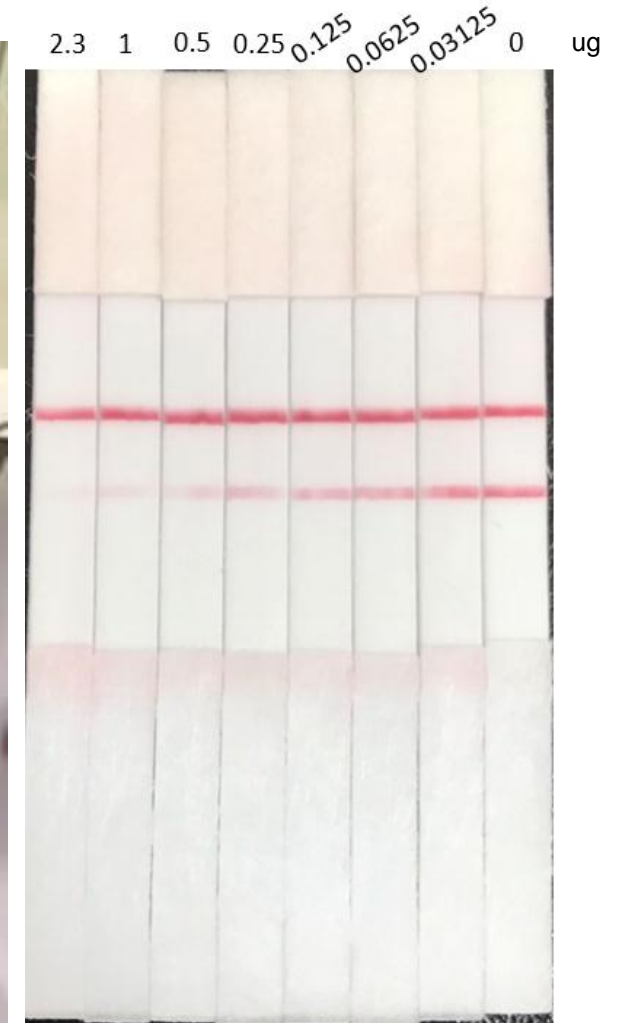
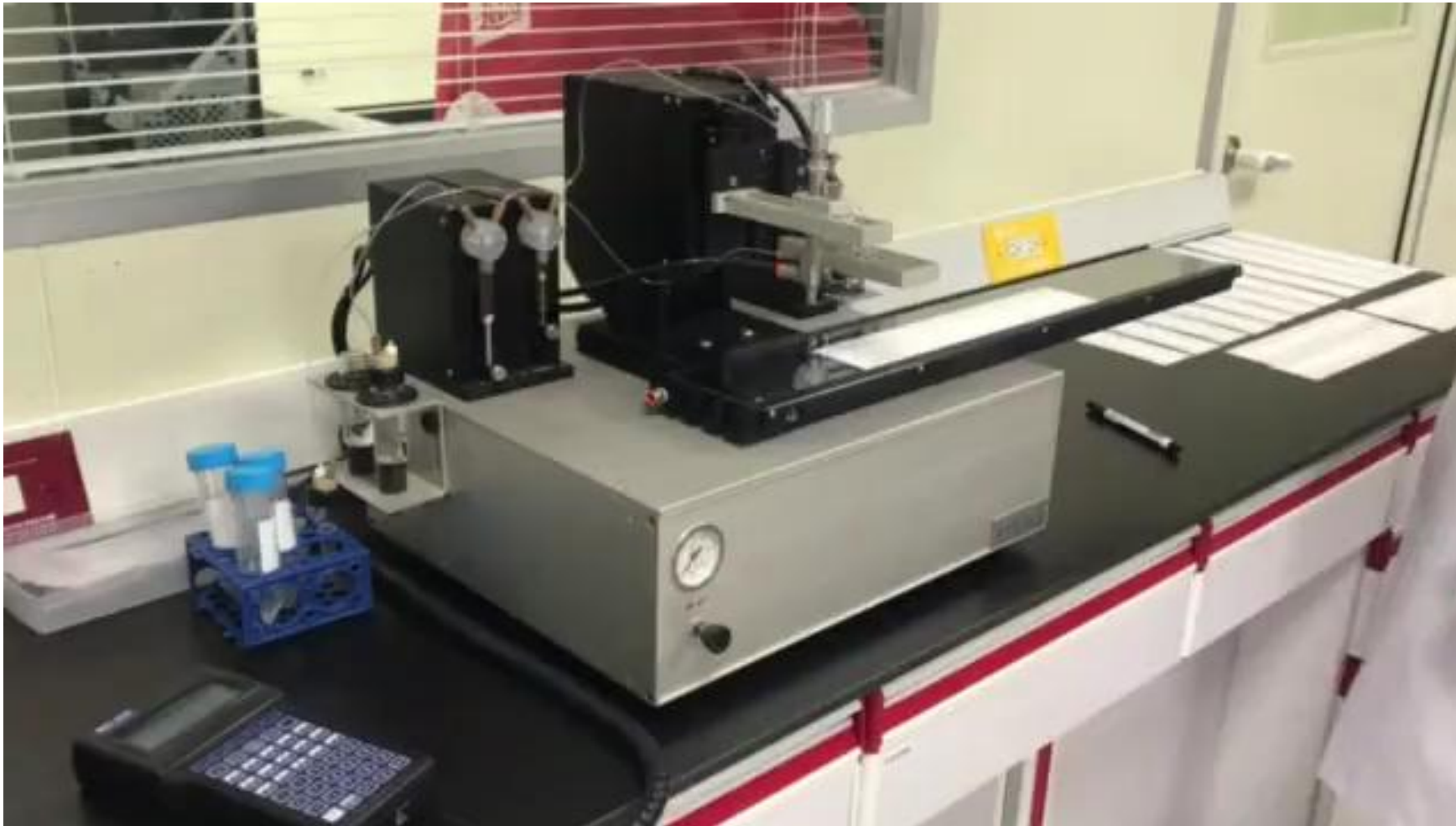
SPA-EGFR<sup>858</sup>-Pol II-GLK Tg mice (Fig. 1D). We next studied whether GLK transgene induces lung cancer (EGFR<sup>858</sup>-positive) metastasis to other organs in SPA-EGFR<sup>858</sup>-Pol II-GLK Tg mice. We performed IHC using anti-EGFR deletion-mutant antibodies on the tissues of the cervical lymph nodes, the liver, and the brain from wild-type and three different transgenic mice. For regional metastasis to cervical lymph nodes, all but one (14/15) SPA-EGFR<sup>858</sup>-Pol II-GLK Tg mice displayed numerous metastatic EGFR<sup>858</sup>-expressing lung cancer cells in cervical lymph nodes. In

# 百歐抗體製作過程





# 百歐試片條件測試



# 國家衛生研究院檢體申請核可



## 台灣新型嚴重性肺炎研究網 Taiwan Severe Pneumonia Network

Dear Professor Lee,

We are pleased to inform you that your application (TSPN No.20-005) for biosamples: Serum of 50 COVID-19 positive patients, 5 COVID-19 patients from positive to negative, and 5 COVID-19 negative patients and related clinical information from TSPN has been approved by the Scientific Review Committee. Since TSPN is now belonged to the NHRI Biobank, all applications need to be approved by the Ethic and Governance Committee of NHRI Biobank, too. Please provide the following documents to our office for further processing:

1. The approval form from your Institutional Reviewing Board (IRB) to conduct the project (pdf file).
2. The approval form of the funding agency to sponsored the project (pdf file).

After we receive the above documents, your application will be reviewed and discussed in the regular meeting of the Ethic and Governance Committee to get the final approval.

Please be noted that, though the biosample itself is free, we do charge "processing fee" to cover the expense for specimen preparation. If you have any concern or question, please feel free to contact us.

Taiwan Severe Pneumonia  
Network Office

台灣新型嚴重性肺炎研究網

2020.05.29

Taiwan Severe Pneumonia Network

國家衛生研究院 苗栗縣竹南鎮科研路 35 號 國家衛生研究院行政大樓 A-3120 室  
National Health Research Institutes, 35, Keyan Road, Zhunan Town, Miaoli County, Taiwan 350  
Telephone: 886-37-206166 ext 33327, Fax: 886-37-583109

## 台灣新型嚴重性肺炎研究網 Taiwan Severe Pneumonia Network

Dear Doctor Lee,

We are pleased to inform you that your application (TSPN No.20-010) for biosamples: Serum of 5 COVID-19 positive patients, 5 COVID-19 negative patients, and 70 COVID-19 negative patients with antibody of Influenza A/B or Adenovirus or Respiratory syncytial virus and related clinical information from TSPN has been approved by the Scientific Review Committee. Since TSPN is now belonged to the NHRI Biobank, all applications need to be approved by the Ethic and Governance Committee of NHRI Biobank, too. Please provide the following documents to our office for further processing:

1. The Institutional Reviewing Board (IRB) approval form or the proof of IRB application to conduct the project (pdf file).
2. The approval form of the funding agency to sponsored the project (pdf file).

After we receive the above documents, your application will be reviewed and discussed in the regular meeting of the Ethic and Governance Committee to get the final approval.

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Taiwan Severe Pneumonia  
Network Office

台灣新型嚴重性肺炎研究網

2020.07.13

Taiwan Severe Pneumonia Network

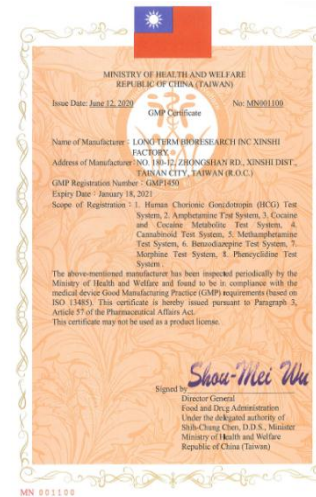
國家衛生研究院 苗栗縣竹南鎮科研路 35 號 國家衛生研究院行政大樓 A-3120 室  
National Health Research Institutes, 35, Keyan Road, Zhunan Town, Miaoli County, Taiwan 350  
Telephone: 886-37-206166 ext 33327, Fax: 886-37-583109



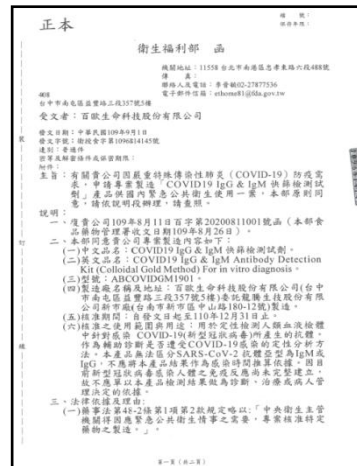
# 實驗室認證與產品認證



TAF 認證實驗室



GMP製造廠



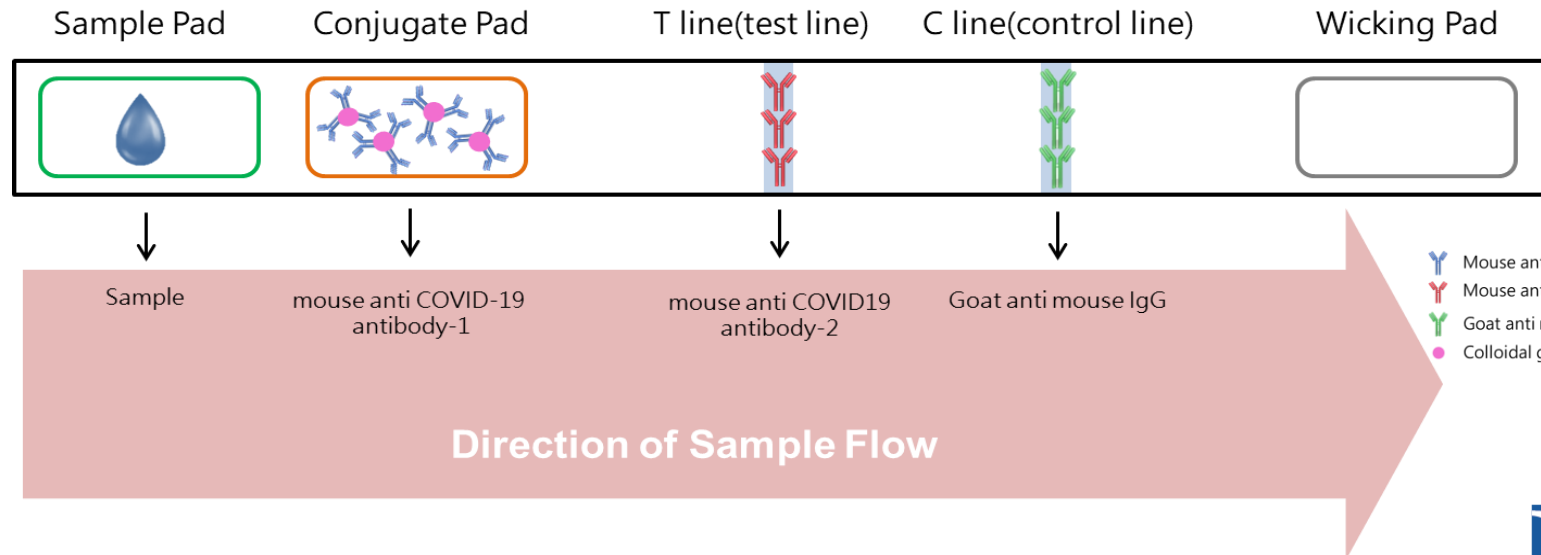
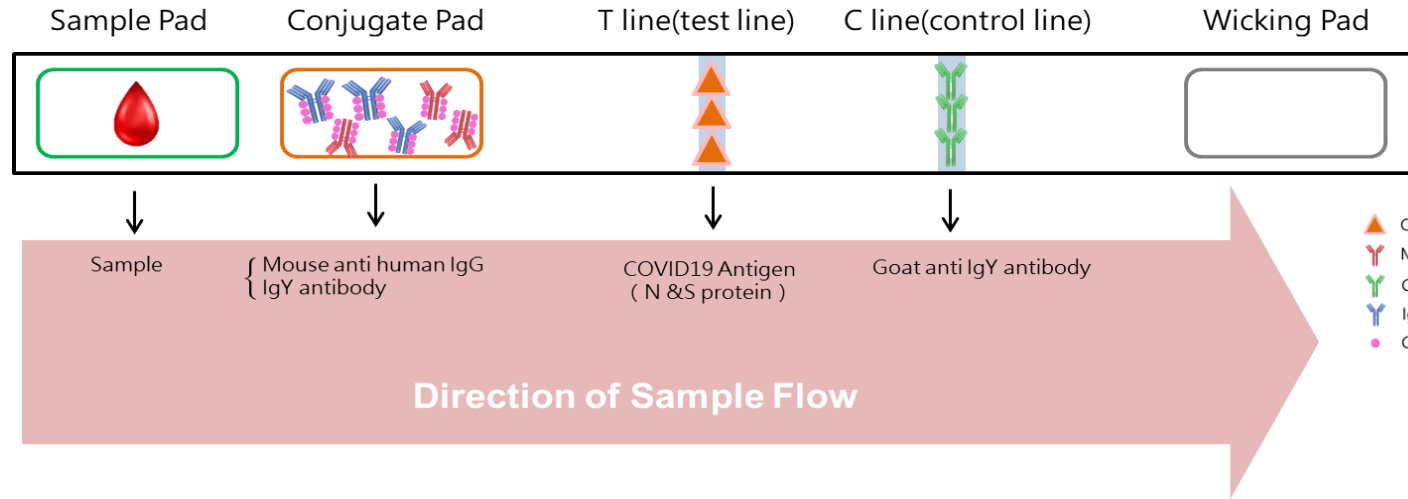
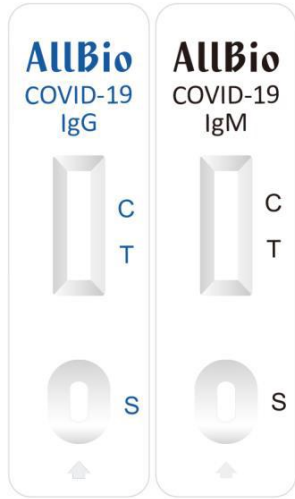
TFDA防疫專案核准製造  
第1096814145號



CE認證



# 快篩設計原理



# 抗體快篩實測結果

Sample source : NHRI Biobank

Number : No. 3

Result : Negative



Number : No. 16

Result : Positive



Stage : 通報確診時

Number : No. 56

Result : Positive



Stage : 住院康復期

Number : No. 74

Result : Negative



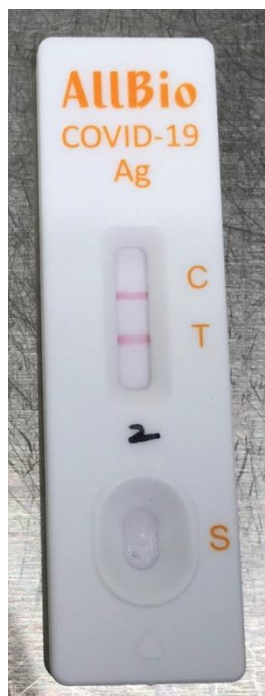


# 抗原快篩實測結果

Sample source : Taoyuan General Hospital

Number : No. 2

Result : **Positive**



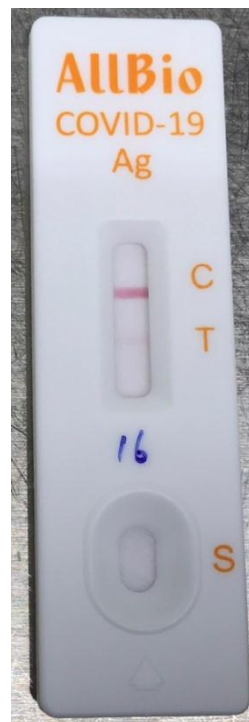
Number : No. 9

Result : Negative



Number : No. 16

Result : **Positive**



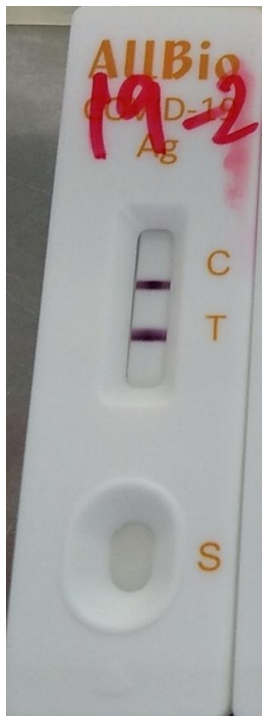
Number : No. 19

Result : **Positive**

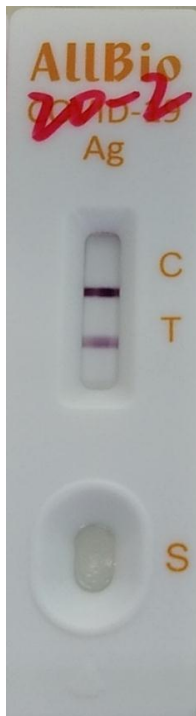


# 抗原快篩實測結果

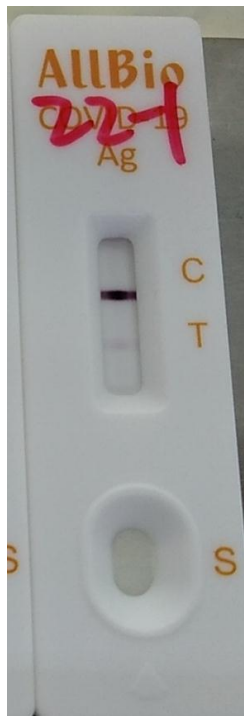
Sample source : Taoyuan General Hospital



CT: 16.6  
**Positive**



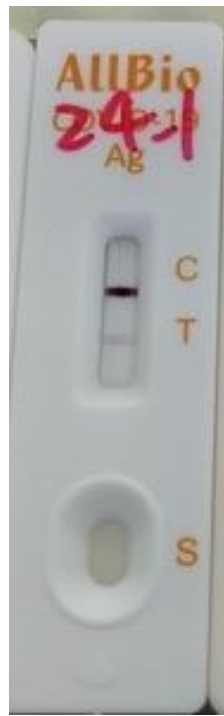
CT: 13.6  
**Positive**



CT: 13.1  
**Positive**



CT: 16.6  
**Positive**



CT: 24.9  
**Positive**



**Negative**

# COVID-19快篩試劑產品



**AllBio**

## COVID-19 IgG & IgM Detection Kit

**COVID-19 IgG & IgM Detection Kit**

8 minutes is ok!

- » High sensitivity and specificity
- » Rapid Test Time (5~8 Mins)
- » Simple detection of COVID-19 IgG & IgM

**AllBio COVID-19 IgG & IgM Detection Kit**

Intended Use: Detection of COVID-19 IgG & IgM

Package: 1 Test / kit

Storage: 2~30°C

Specimen Type: Whole blood / Fingertip blood / Serum / Plasma

Shelf life: 18 months from manufacture

Time to result: 5~8 minutes



**AllBio**

## COVID-19 Ag Rapid Test Kit

**COVID-19 Antigen test**

8 minutes is ok!

- » High sensitivity and specificity
- » Rapid Test Time (5~8 Min)
- » Simple detection of COVID-19 antigen

**AllBio COVID-19 Ag Rapid Test Kit**

Intended Use: Detection of COVID-19 antigen

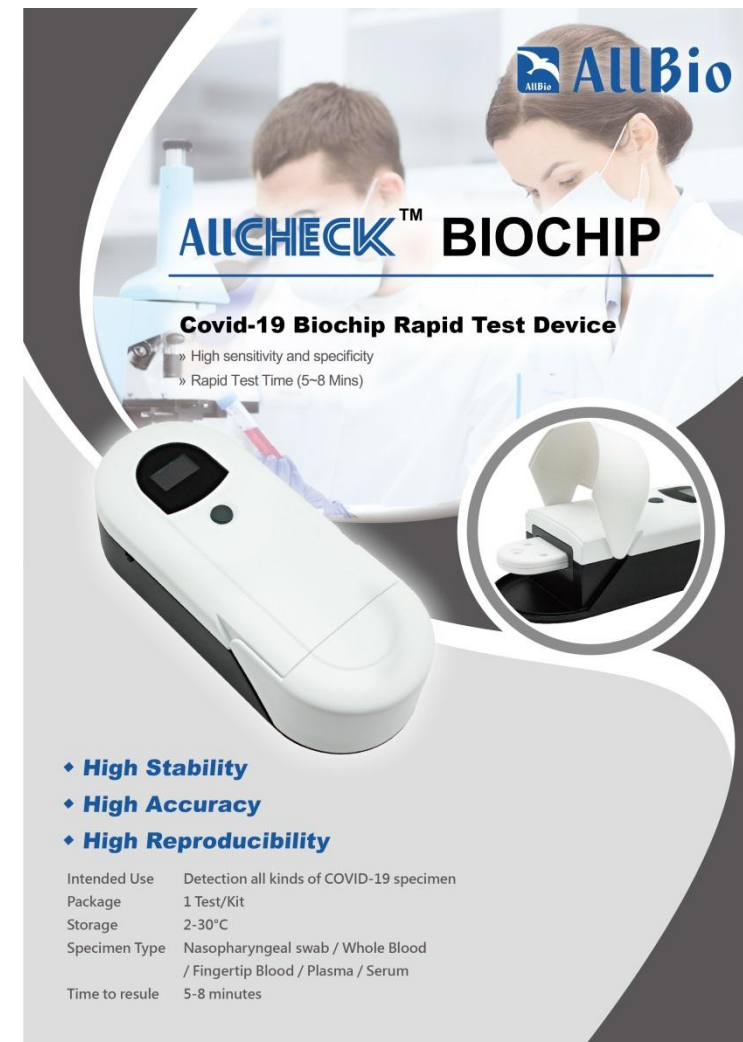
Package: 20 Tests / kit

Storage: 2~30°C

Specimen Type: Nasopharynx / Nasopharyngeal

Shelf life: 18 months from manufacture

Time to result: 5~8 minutes



**AllBio**

## AUCHECK™ BIOCHIP

**Covid-19 Biochip Rapid Test Device**

8 minutes is ok!

- » High sensitivity and specificity
- » Rapid Test Time (5~8 Mins)

**AllBio COVID-19 Ag Rapid Test Kit**

Intended Use: Detection all kinds of COVID-19 specimen

Package: 1 Test/Kit

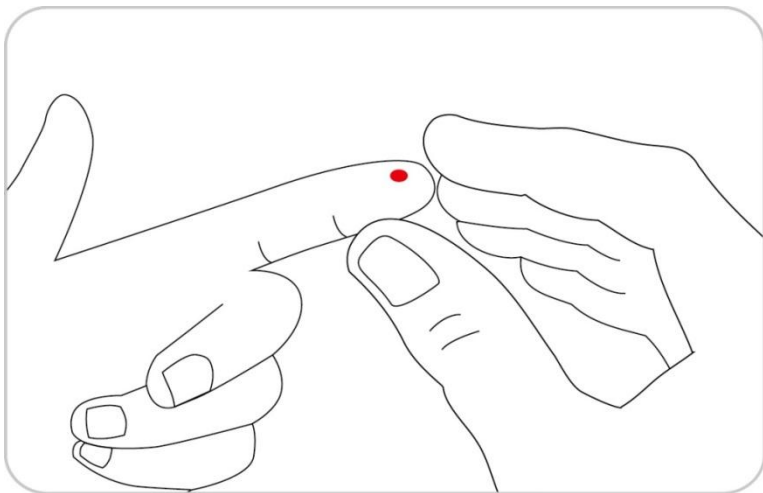
Storage: 2~30°C

Specimen Type: Nasopharyngeal swab / Whole Blood / Fingertip Blood / Plasma / Serum

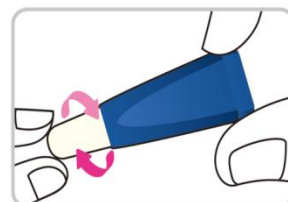
Time to result: 5~8 minutes



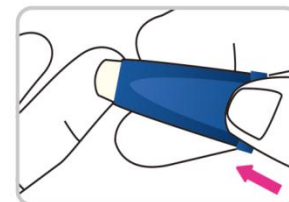
# 百歐快篩試劑操作流程



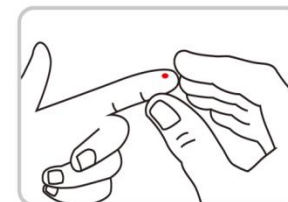
1. 進行採血步驟前，請先以酒精棉片消毒採血部位並保持乾燥。



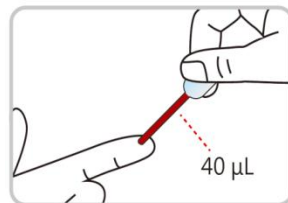
2. 旋開無菌帽蓋。



3. 將採血器緊貼在選定的採血點上，以拇指完全向前釋放按壓採血器。



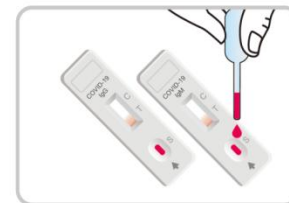
4. 適當的以拇指按壓採血點，以便採集足夠的血液樣本。



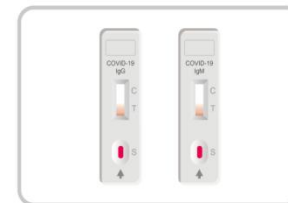
5. 用滴管收集血液樣本。



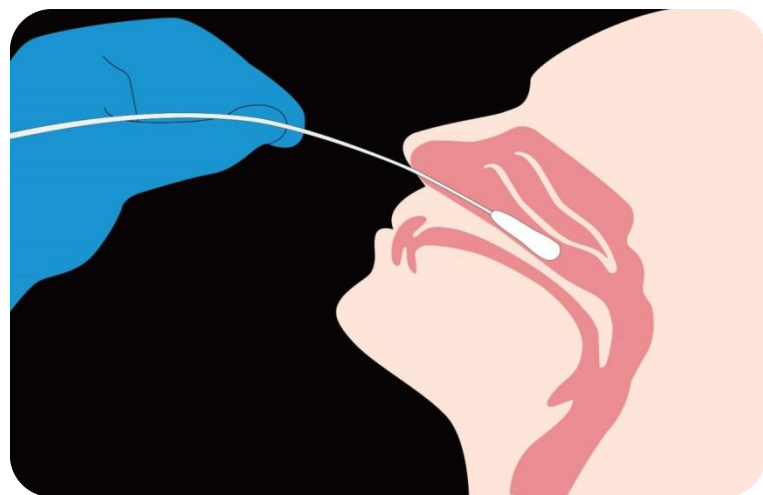
6. 用滴管將血液樣本與稀釋液混合均勻。



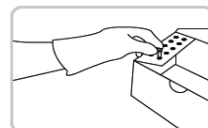
7. 用滴管滴加 2 滴混合好之樣本到檢測卡匣的樣本槽中，注意避免氣泡產生。



8. 等待 5-8 分鐘讀取檢測結果，若 20 分鐘後質量控制線 (C) 未呈現深紅色條帶反應，則為無效結果，應將其丟棄。



1 Open the cap of the diluent tube.



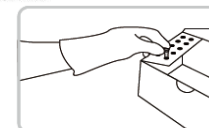
2 Insert the diluent tube into the fixing hole of the box.



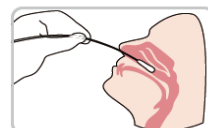
3 Open the cap of the Chase buffer.



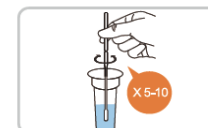
4 Add 10 drops (300ul) of Chase buffer



5 Insert the diluent tube into the fixing hole of the box.



7  
Nasopharyngeal specimen is preferred for swab-based COVID-19 testing. If the nasopharyngeal specimen is not available, then any of the following is acceptable:  
• Oropharyngeal specimen collected by a healthcare professional (HCP)  
• Mid-turbinate specimen\* by onsite self-collection or HCP (using a flocked tapered swab)  
• Anterior nares specimen\* by onsite self-collection or HCP (using a round foam or polyester swabs).  
\* At this time, anterior nares and mid-turbinate specimen collection is only appropriate for symptomatic patients and both nares should be swabbed.



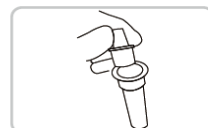
8 Insert the swab specimen and swirl the swab 5-10 times.



9 Remove the swab while gently squeezing the head of the swab.



11 Place the nasopharyngeal swab in the biohazard bag.



12 Close the diluent tube with a filter cap securely.



13 Invert the diluent tube and gently squeeze it to draw 3-4 drops (90 ~ 150ul) into a specimen well on the device.



14 Place the diluent tube in the biohazard bag.

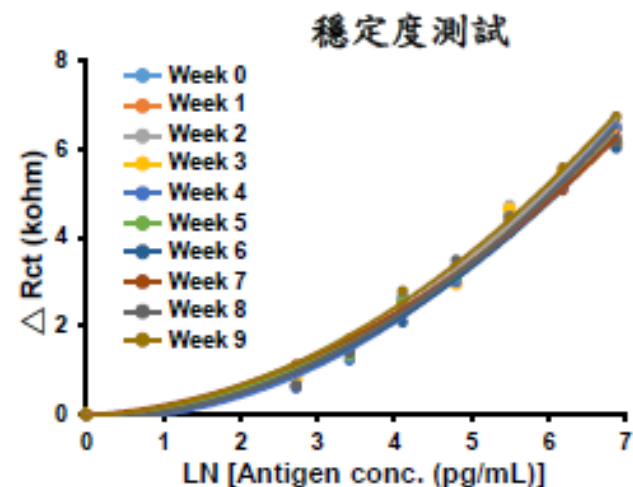
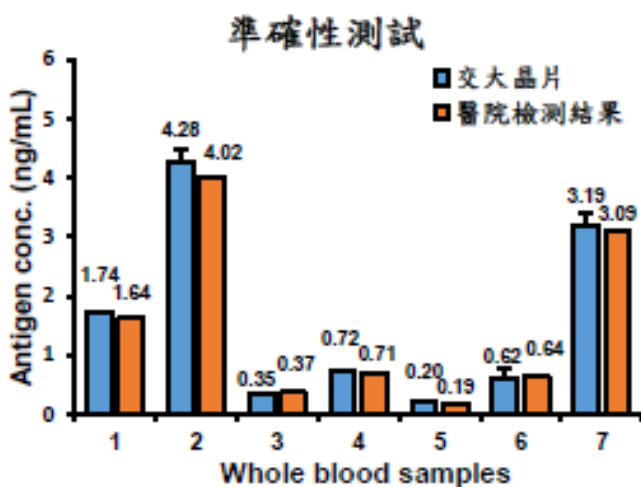
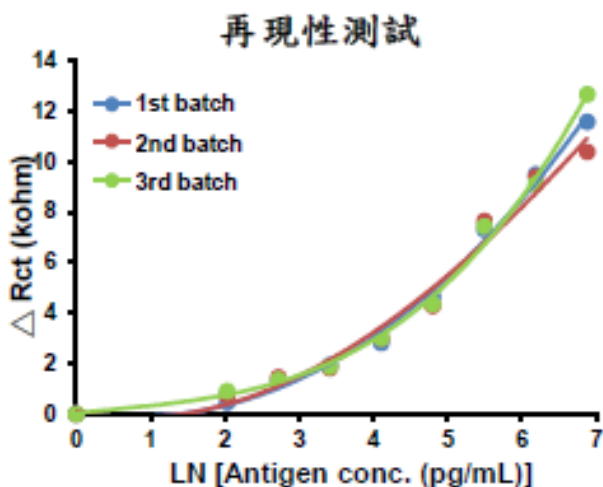


15 Read the result within 5-8 minutes.

# 組合式電晶片平臺-新冠肺炎晶片檢測系統



# AIICHECK 快篩晶片平台性能測試



	再現性測試	準確性測試	穩定度測試
說明	每批次穩定度高	檢測結果與現品一致	可長期保存
結果	再現性 > 96%	準確性 > 95%	穩定度 > 94%
結論	具備量產能力	具備高準確度檢測結果	具備長時間有效保存

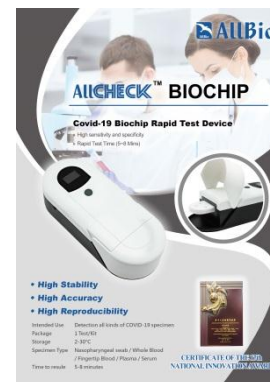
Chen LC, Wang Erick, Tai CS, Chiu YC, Li CW, Lin YR, Lee TH, Huang CW, Chen JC, Chen WL\*.

Improving the reproducibility, accuracy, and stability of an electrochemical biosensor platform for point-of-care use.

Biosensors and Bioelectronics 155 (2020): 112111. DOI: 10.1016/j.bios.2020.112111 (IF= 10.257)



# COVID-19檢測平台比較



檢測方式	抗體快篩檢測	抗原快篩檢測	生物晶片平台	即時聚合酶鏈鎖反應
原理	檢測抗體	檢測抗原	可檢測抗體或抗原	檢測病毒RNA
設備	不需要	不需要	可攜式手持裝置	PCR 機器
場地	不需要特定場域	不需要特定場域	不需要特定場域	P2 plus or P3 實驗室
檢體	血液 (全血、指尖血、血漿、血清)	鼻咽、咽喉、下呼吸道檢體	不受限	鼻咽、咽喉、下呼吸道檢體
檢測結果	方便判讀	方便判讀	方便判讀 可雲端紀錄追蹤	需要專業人員判讀實驗數據
所需時間	快速(5-8分鐘)	快速(5-8分鐘)	快速(5-8分鐘)	8-9 小時

# Thanks for Your Listening



# AllBio