

Information

Chip Hybridization. For convenience and high signal reliability, hybridization is best performed using a CapitalBio BioMixer™ II Microarray Hybridization Station and HybSet™ Microarray Hybridization Cassette which both help to reduced edge-effects. The enhanced quality of hybridization is attested in recent publications such as Patterson *et al* (2006) *Nature Biotechnology* 24:1140-1150 and Shi *et al* (2006) *Nature Biotechnology*, 24:1151-1161.

Ordering Information

Cat. No.	Product Name	Product Description
301030	CapitalBio <i>Mycobacteria</i> Identification Array Kit	24 tests

For research use only

CapitalBio Corporation

18 Life Science Parkway
Changping District
Beijing 102206
P. R. China
Tel: 86-10-80726868
Fax: 86-10-80726782
globalsales@capitalbio.com
www.capitalbio.com



CapitalBio *Mycobacteria* Identification Array Kit

Cat. No. 301030

User Manual

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If tuberculosis disease is suspected, use of one or more additional analysis methods is recommended to confirm the test results.

3. Contents of the Kit

- 3.1 Six chips, packed in a plastic box. Each chip contains 4 identical blocks.
- 3.2 SmartCover™ cover slips (coded No.11, 6 pieces, packed together with the chips in the same plastic box).
- 3.3 Decontamination Buffer, 1 vial (coded No.1), 80 ml.
- 3.4 DNA Washing Buffer, 1 vial (coded No.2), 30 ml.
- 3.5 DNA Extraction Buffer, 1 vial (coded No.3), 5 ml.
- 3.6 DNA Extraction Tubes (coded No.4), 24 tubes.
- 3.7 PCR Amplification Mix, 1 vial (coded No.5), 600 µl.
- 3.8 Positive Control DNA (for Identification), 1 vial (coded No.6), 20 µl.
- 3.9 Negative Control DNA (for Identification), 1 vial (coded No.7), 20 µl.
- 3.10 Hybridization Buffer, 1 vial (coded No.8), 300 µl.
- 3.11 20 × SSC, 1 vial (coded No.9), 140 ml.
- 3.12 10% SDS, 1 vial (coded No.10), 30 ml.

	1	2	3	4	5	6	7	8	9	10
1	QC					EC				
2	BC					Mycobacterium genus				
3	<i>M. tuberculosis</i>					<i>M. intracellulare</i>				
4	<i>M. avium</i>					<i>M. gordonae</i>				
5	<i>M. kansasii</i>					<i>M. fortuitum</i>				
6	<i>M. scrofulaceum</i>					<i>M. gilvum</i>				
7	<i>M. terrae</i>					<i>M. chelonae-M. abscessus</i>				
8	<i>M. phlei</i>					<i>M. nonchromogenicum</i>				
9	<i>M. marinum-M. ulcerans</i>					<i>M. aurum</i>				
10	<i>M. szulgai</i>					<i>M. xenopi</i>				
11	<i>M. smegmatis</i>					NC				
12	EC					QC				

Fig.1 Array layout (each block contains 17 species-specific probes and 4 controls)

- QC:** Chemical control (array production control)
- EC:** External control (hybridization control)
- BC:** Blank control
- NC:** Negative control

8.2.6. Preparation of hybridization reaction

Prepare the appropriate number of 200 µl microtubes or 8-strip tubes and mark with a specimen code.

Thaw out the Hybridization Buffer (Tube No.8) completely at 50°C in the incubator. Mix contents well and centrifuge at 4,000 rpm for 15s. Aliquot 9 µl of each mixture into the microtubes, or the 8-strip tubes. Add 6 µl of PCR product to each tube to make up a final volume of 15 µl. The hybridization reaction mixes are denatured at 95°C for 5 min (in a PCR instrument or incubator). Then immediately put the tubes onto the ice-water mix for 3 min.

8.2.7 Hybridization reaction

Pipette hybridization reaction mixes several times until the precipitate disappears. Transfer 13.5 µl of each mix to the chip surface through the sample port of the SmartCover cover slip. Close the hybridization cassette. Record the chip code and block location.

Place the closed hybridization cassette into the incubator at once and incubate at 50°C for 120 min (count the time from the placing of the last cassette into the incubator).

8.3 Washing

8.3.1 Open the hybridization cassette, discard the cover slips and transfer the chips to the chip rack soaked in **Wash Buffer 1**.

8.3.2 Place the rack filled with chips in the bench-top shaker and shake at 80 rpm for 3 min.

8.3.3 Transfer the rack filled with chips to **Wash Buffer 2** and shake at 80 rpm for 3 min.

8.4 Drying

Transfer each of the chips into a CapitalBio Slide Centrifugation Tube. Cap the tubes and centrifuge at 1,000 rpm for 1 min to dry the chips.

8.5 Data Acquisition

After washing and drying, the chips should be stored at room temperature and analyzed as soon as possible to avoid fluorescence quenching. Scan the chip using a LuxScan™ 10K-B Microarray Scanner. The results are generated automatically according to the instructions of the “CapitalBio *Mycobacteria* Identification Array Test System”. If the chip cannot be analyzed immediately, it should be stored in dark and dry conditions, and analyzed within 24 hours.

6. Materials required but not provided

6.1 Equipments

- CapitalBio LuxScan™ 10K-B Microarray Scanner (Cat. No. 100020)
- CapitalBio BioMixer™ II Microarray Hybridization Station (Cat. No. 120030, optional)
- CapitalBio HybSet™ Microarray Hybridization Cassette (Cat. No. 430010)
- CapitalBio Slide Centrifugation Tubes: 6
- PCR instrument
- Centrifuge (>10,000 rpm, for 1.5 ml microtubes)
- Centrifuge (for drying washed chips)
- Temperature-controlled incubator (optional)
- Bench-top incubation shaker
- Vortex mixer
- Laboratory timer
- Container for ice and water mix
- Chip washing boxes: 2
- Chip rack (optional)
- Measuring cylinders: 25 ml × 1 and 500 ml × 1
- Reagent bottles: 1000 ml × 3

6.2 Others

- Micropipettes for 10 µl, 100 µl and 1000 µl
- Clean pipette tips for 10 µl, 100 µl and 1000 µl micropipettes
- Sterilized 8-strip 200 µl tube and caps (optional)
- Sterilized 200 µl and 1.5 ml microtubes
- Tube rack for 200 µl and 1.5 ml microtubes

6.3 Reagent Preparation:

- Distilled or deionized water
- Water-ice mixture

1. Introduction

CapitalBio *Mycobacteria* Identification Array Detection Kit is used for the detection of 17 most frequently encountered *Mycobacterial* species, including *M. tuberculosis* complex, *M. intracellulare*, *M. avium*, *M. gordonae*, *M. kansasii*, *M. fortuitum*, *M. scrofulaceum*, *M. gilvum*, *M. terrae*, *M. chelonae*, *M. abscessus*, *M. phlei*, *M. nonchromogenicum*, *M. marinum*, *M. ulcerans*, *M. aurum*, *M. szulgai*, *M. xenopi* and *M. smegmatis*. Genomic DNA is extracted for test, either from isolated strain culture or directly from clinical sputum samples. The whole detection assay takes less than 6 hours, which is notably shorter than biochemical assays that require at least two weeks. The *Mycobacteria* Identification Array Detection Kit has a combination of special primer designs, strictly controlled PCR procedure, standard array hybridization process and automatic software analysis providing high-throughput personalized therapy advice. The test results from this kit serve as a reference for clinicians when a diagnosis is being made. A diagnosis cannot be made based only on the results obtained from this test kit.

2. Principle of the Test

The 16S rDNA-specific primers and the corresponding species-specific oligonucleotide probes are designed based on the sequence of the 16S rDNA gene. The oligonucleotide probes are immobilized on the chip surface to recognize different species.

The DNA extracted from clinical isolates or specimens is used as a template for PCR. Because the primer is tagged with fluorescence molecules, the amplified PCR products obtained are fluorescently tagged. Then the PCR products are used for microarray hybridization in the hybridization buffer provided. After washing and drying, the chips are scanned using a microarray scanner. The interpretation software of the array kit is then used to analyze the scan data and to generate the test reports, based on the distribution of positive probe signals on the microarray.

11. Troubleshooting

Problem	Likely Cause	Solution
<ul style="list-style-type: none"> No signal, including QC probe 	<ul style="list-style-type: none"> Chip inserted in the wrong direction while scanning Damage to the chip Scanner or software failure 	<ul style="list-style-type: none"> Adjust the direction of the chip when scanning Scan a control chip that worked well before
<ul style="list-style-type: none"> Only QC signal is present, but EC signal is absent 	<ul style="list-style-type: none"> Hybridization failed. Incorrect manipulation of the hybridization reaction Incorrectly prepared chip wash buffer 	<ul style="list-style-type: none"> Check the chip code and the experiment report Check the hybridization temperature Check chip wash buffer
<ul style="list-style-type: none"> Positive signal present in blank or negative control 	<ul style="list-style-type: none"> Dust present on the chip surface Hybridization solution vaporized, generating high background Incorrectly prepared chip wash buffer Contamination of sample / reagents / pipettes 	<ul style="list-style-type: none"> Ensure clean area Work quickly with Hybridization solution Check wash buffer Decontaminate Pre-PCR clean area and PCR staging area including all equipment. Use new reagents and new pipettes.
<ul style="list-style-type: none"> Positive signal present in QC and EC control probe, but all other probes show no signal in both samples and positive control. 	<ul style="list-style-type: none"> PCR failure Sample DNA contains enzymatic or chemical inhibitors 	<ul style="list-style-type: none"> Check PCR instrument and program. Check PCR products by gel electrophoresis Ensure complete removal of bacterial sediment when extracting DNA samples

4. Storage and stability

The kit (A) should be stored at room temperature and the kit (B) should be stored at -20°C. The kit remains stable for at least 6 months from the date of manufacture if handled properly. Do not use the kit after the stated expiry date. If the vials are opened, the kit should be used within one month. The kit is stable during shipment under recommended shipping temperature conditions.

5. Warnings and precautions

- This kit is for *in vitro* use only.
- **Pathogen:** Wear exposure suit, mask and disposable gloves while handling specimens, and any material or equipment must be sterilized before reuse or destroyed if polluted by bacterial growth or specimens.
- The reagents should be mixed thoroughly prior to use and repetitive thawing and freezing should be avoided.
- **For all PCR steps:** sample preparation, reaction mix preparations and addition of templates should be performed in separate places to avoid false positive signals resulting from contamination.
- Use filtered pipette tips with this PCR assay kit. This precaution can prevent DNA aerosol contamination.
- The chips, the PCR amplification mix and the hybridization buffer are all light sensitive. Do not expose them to strong light sources.
- Bring chips to room temperature (20-25°C) prior to use.
- Cover slips are one-time use only.
- Avoid generating air bubbles when adding sample solutions onto the chip surface.
- Incomplete or inadequate washing of the chip may cause high background or false positive results.
- Avoid touching or contacting the arrayed area.

9. Quality control

The positive control and negative control should be run with every chip to ensure that all reagents and procedures performed properly. Expected values for each control are shown in **Table 3**.

Table 3. Expected values for quality control

Control	Expected value
NC	≤500
BC	≤200
QC	≥5000
EC	≥3000

10. Interpretation of results

The purpose of the CapitalBio *Mycobacteria* Identification Array Kit is to assist physicians to make a clinical diagnosis for tuberculosis diseases.

Probe positive: A positive result is the signal to noise ratio of one oligonucleotide probe ≥ 3 and \geq its cutoff value.

Probe negative: A negative result is the signal to noise ratio of one oligonucleotide probe < 3 or $<$ its cutoff value.

Identification interpretation: To detect if any one of the species-specific probes of the 16S rDNA gene is positive or not.

7. Extraction of DNA

- 7.1 Clinical culture and sputum specimens must be handled by a certified medical technologist.
- 7.2 **For culture:** Add 80 µl of DNA extraction buffer into a DNA extraction tube. Pick bacteria culture with a sterilized toothpick or tip and rub at the inner bottom of an Extraction Tube to transfer the culture into the tube. Vortex the Extraction Tube at the highest speed for 5 min by CapitalBio Extractor™ 36. Incubate the Extraction Tube at 95°C for 5 min. Centrifuge and store the extracted DNA at -20°C.
- 7.3 **For sputum specimens:** The specimens are liquefied with an equal volume of Decontamination Buffer for 30 min at 37°C and centrifuged at 10,000 rpm for 5 min. The supernatant is discarded and the sediment is resuspended in 1 ml of DNA Washing Buffer and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and the sediment is resuspended in 80 µl of DNA Extraction Buffer and transferred to a DNA Extraction Tube. Extraction Tube at the highest speed for 5 min by CapitalBio Extractor™ 36. Incubate the Extraction Tube at 95°C for 5 min, centrifuge and store the extracted DNA at -20°C.
- 7.4 The extracted DNA can be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- 7.5 Avoid repetitive freezing and thawing of samples.

8. Assay procedure

8.1 Pre-test preparation

8.1.1 Wash Buffer 1:

Orderly add 20×SSC: distilled water: 10% SDS=10:88:2, then mix well by stirring or gentle shaking before use.

8.1.2 Wash Buffer 2:

Add 10% SDS: distilled water =1:99, then mix well by stirring or gentle shaking before use.

8.1.3 Use a 50:50 ice and water mix for the hybridization procedure.

8.2 Experimental procedure

8.2.1 Determine the total number of tests and prepare the appropriate volumes of reagents from the package.

8.2.2 Preparation of PCR reaction solution

Take an appropriate number of 200 µl microtubes or 8-strip tubes and mark with specimen codes. Take the Amplification Mix (Tube No.5) from the kit and mix gently after thawing. Briefly centrifuge to collect contents to bottom. According to the number of specimens, take the calculated volume of the Amplification Mix. Then distribute 18 µl of the Amplification Mix in the microtubes or 8-strip tubes and cap the tubes. Two microlitres of extracted DNA, **Positive Control** (Tube No.6) or **Negative Control** (Tube No.7) is added to each tube to make up a final volume of 20 µl (**Table 1**).

Table 1. The components of PCR reaction solution

Component	Volume (µl)
PCR Amplification Mix	18.00
Template	2.00
Total	20.00

8.2.3. PCR reaction

Place the microtubes or 8-strip tubes into a PCR instrument. The amplification reaction is accomplished according to the thermocycling program shown in **Table 2**.

Table 2. PCR thermocycling program

Temp (°C)	37	94	94	60	72	94	72	72	4
Time (s)	600	600	30	30	40	30	60	420	-
No. of Cycles	1	1	35			10		1	1

8.2.4. Turn on the incubator and set to 50°C.

8.2.5. Determine the total number of chips needed and take the appropriate number of chips out of the package.

8.2.5. Fill in the hybridization cassette in the base with 200 µl of distilled water to keep high humidity during hybridization. Place the chip into the cassette with the barcode side facing up and the barcode towards the operator. Then cover the chip with the cover slip.