

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
301035	CapitalBio <i>M. Tuberculosis</i> Drug Resistance Detection Array Kit	12 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 *M. Tuberculosis* Drug Resistance Detection Array Kit

Cat. No. 301035

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), 140 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), 12 tubes.
- 3.7 PCR Amplification Mix1 (coded No.5), 1 tube, 300µl.
- 3.8 PCR Amplification Mix2 (coded No.6), 1 tube, 300µl.
- 3.9 PCR Amplification Mix3 (coded No.7), 1 tube, 300µl.
- 3.10 Positive Control, 1 vial (coded No.8), 20 µl.
- 3.11 Negative Control, 1 vial (coded No.9), 20 µl.
- 3.12 Hybridization Buffer, 1 tube (coded No.10), 300 µl.
- 3.13 20 × SSC, 1 tube (coded No.11), 140 ml.
- 3.14 10% SDS, 1 tube (coded No.12), 30 ml.

	1	2	3	4	5	6	7	8	9	10
1	QC					EC				
2	BC					<i>rpoB</i> -IC				
3	<i>Mycobacterium spp.</i>					<i>M. tuberculosis</i>				
4	511-WT					511T→C				
5	513-WT					513C→A				
6	516-WT					516A→T				
7	533-WT					533T→C				
8	531-WT					531C→T				
9	526-WT					526C→A				
10	526C→T					526C→G				
11	526A→T					526A→G				
12	516A→G					NC				
13	EC					QC				

Fig.1. Array Layout of *rpoB* gene detection

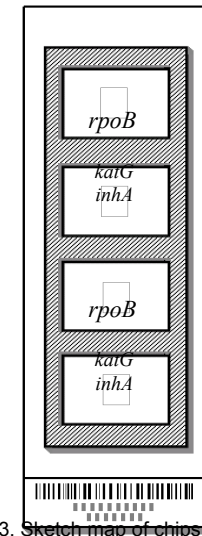


Fig3. Sketch map of chips

Thaw out the Hybridization Buffer (Tube No. 5) completely at room temperature. Mix contents well and centrifuge at 4,000 rpm for 15s. Aliquot 9 μ l of each mixture into the microtubes, or the 8-strip tubes. Add 3 μ l of PCR product 1 and 3 μ l of PCR product 2 (product of *rpoB* gene)/3 (product of *katG* and promoter *inhA* gene) to each tube to make up a final volume of 15 μ l. 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 μ l of each mix to the chip surface through the sample port of the SmartCover cover slip. (The mixture of PCR product 1 and product 2 added to block 1 or block 3, The mixture of PCR product 1 and product 3 added to block 2 or block 4) 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 *Mycobacterial tuberculosis* Drug Resistance Detection 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 \times 1 and 500 ml \times 1
- Reagent bottles: 1000 ml \times 3

6.2 Others

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

6.3 Reagent Preparation:

- Solution A
- Solution B
- Solution C
- Distilled or deionized water
- 20 \times SSC
- 10% SDS

1. Introduction

CapitalBio *M. Tuberculosis* Drug Resistance Array Detection Kit is used for the detection of some frequently observed sites mutations of three genes which strongly implicate resistance to two important first-line anti-tuberculosis drugs (Rifampicin and Isoniazid). 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 *M. Tuberculosis* Drug Resistance 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 MTB-specific primers and the corresponding oligonucleotide probes are designed based on the mutations of the *rpoB* gene (conferring resistance to rifampin), *katG* gene and promoter of *inhA* gene (conferring resistance to Isoniazid). The oligonucleotide probes are immobilized on the chip surface to recognize different mutations.

The DNA extracted from clinical isolates or specimens is used as a template for asymmetric PCR. Because the primer is tagged with fluorescence molecules, the amplified single chain PCR products obtained are fluorescently tagged. Then the PCR products are used for microarray hybridization in the reaction 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

	1	2	3	4	5	6	7	8	9	10
1	QC					EC				
2	BC					BC				
3	<i>Mycobacterium spp.</i>					<i>M. tuberculosis</i>				
4	KatG-IC					InhA-IC				
5	KatG -WT					InhA-WT				
6	KatG 315G→C					InhA-15C→T				
7	KatG 315G→A					NC				
8	EC					QC				

Fig.2. Array Layout of *inhA* and *katG* gene.

QC: Chemical control (array production control)
EC: External control (hybridization control)
BC: Blank control **NC: Negative control**
IC: Internal control **WT: Wild tipe**

4. Storage and stability

The kit 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 (-20°C).

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, 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 reaction 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
BC	≤500
QC	≥3000
EC	≥3000

10. Interpretation of results

The purpose of the CapitalBio *Mycobacterial tuberculosis* Drug Resistance Detection Array Kit is to assist physicians to make a clinical diagnosis for tuberculosis diseases.

Probe positive: A positive result is the signal value of one oligonucleotide probe $\geq BC+3 \times SD$ and ≥ 1000 , and the ratio between the signal of this probe and that of other probes at the same codon site ≥ 1 .

Probe negative: A negative result is the signal value of one oligonucleotide probe $< BC+3 \times SD$ or < 1000 , or the ratio between the signal of this probe and that of other probes at the same codon site < 1 .

Susceptibility interpretation:

Mutant genotype: To detect if any one of the mutation probes of the associated gene is positive or not;

Wild genotype: To detect if all the wild type probes of the associated gene are positive or not.

Table 4. Drug resistance and associated genes.

Gene	<i>rpoB</i>	<i>katG</i>	<i>InhA</i>
Drug	Rifampicin	Isoniazid	

7. Specimen collection, storage and handling

- 7.1 Clinical isolate growth and sputum specimens must be handled by a certified medical technologist.
- 7.2 **For plate culture:** Pick single bacteria colony on the plate with a sterilized toothpick or tip. Rub at the inner bottom of an Extraction Tube to transfer the bacteria colony into the tube. Add 50 µl of DNA Extraction Buffer.
- 7.3 **For sputum specimens:** The specimens are liquified with an equivalent volume of solution A for 30 min at room temperature and centrifuged at 10,000 rpm for 5 min. The supernatant is discarded and the sediment is resuspended in 1 ml of solution B and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded and the sediment is resuspended in 50 µl of solution C and transferred to an Extraction Tube. (The required materials and reagents are supplied in the CapitalBio Universal Kit for Bacterial DNA Extraction).
- 7.4 Vortex the Extraction Tube at the highest speed possible for 5 min. CapitalBio Extractor™ 36 is designed to process small volume DNA extractions in 36×1.5 ml microtubes and is recommended for this assay.
- 7.5 Incubate the Extraction Tube at 95°C for 5 min.
- 7.6 The extracted DNA can be refrigerated at 2-8°C for up to five days or stored at –20°C up to six months.
- 7.7 Avoid repetitive freezing and thawing of samples.

8. Assay procedure

8.1 Pre-test preparation

8.1.1 Wash Buffer 1:

For up to 16 chips, add 60 ml of 20×SSC and 528 ml of distilled water into a 1000 ml reagent bottle and mix thoroughly. Then add 12 ml of 10% SDS to make up a final volume of 600 ml. Mix well by stirring or gentle shaking before use.

8.1.2 Wash Buffer 2:

For up to 6 chips, add 6 ml of 20×SSC and 594 ml of distilled water into a 1000 ml reagent bottle to make up a final volume of 600 ml. 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 Tube No.5-No.9 from the kit and mix gently after thawing. Briefly centrifuge to collect contents to bottom.

According to the number of specimens, take the volume of **PCR Reaction Mix** 18 µl/reaction tube (Tube No.5-7) at the ratio shown in **Table 1**. Then distribute 18 µl of the mixture to each in the microtubes or 8-strip tubes and cap the tubes.

Transfer the microtubes or 8-strip tubes to the template area for PCR. Two microlitres of extracted DNA, **Positive Control** or **Negative Control** is added to each tube to make up a final volume of 20 µl.

Table 1. The components of PCR reaction solution

Component	Volume (µl)
PCR Reaction Mix1/2/3	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 200µl of distilled water to keep it humid. 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.
- 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.