



# b-CAP assay

For the detection of bacterial Community Acquired Pneumonia using the BD MAX™ system

Instructions for use

*Distributed by*





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## Introduction

This protocol describes the system settings and run setup protocol for the detection of bacterial Community Acquired Pneumonia caused by *Chlamydomphila pneumoniae* (Cpn), *Legionella pneumophila* (Lpn), *Chlamydia psittaci* (Cps) and *Mycoplasma pneumoniae* (Mpn) using the BD MAX system.

The assay targets the following genes:

Target	Gene
Cpn	Outer membrane protein A gene
Lpn	Macrophage infectivity potentiator protein gene
Cps	Outer membrane protein A gene
Mpn	P1 adhesion protein gene

The b-CAP assay was optimized for the BD MAX system by PAMM laboratories, Veldhoven. Validation was performed on throat-swabs (E-swab, Copan), BAL and sputum samples.

## Contact information

For information regarding ordering dried snap-in tubes for the b-CAP assay:  
[info@biolegio.com](mailto:info@biolegio.com)

For information regarding to the protocol:  
[secretariaatMM@pamm.nl](mailto:secretariaatMM@pamm.nl)



## 1. Protocol

This protocol describes the assay settings that are required for a to run the b-CAP assay on the BD MAX system. The b-CAP snap-ins contain primers and probes for the detection of Cpn, Lpn, Cps and Mpn and require no further preparation. For monitoring failure in extraction procedure an internal Sample Process Control (SPC) is present in the BD MAX Sample Buffer tube which is detected using the BD MMK (SPC) mastermix.

### 1.1 Materials needed

- BD MAX instrument
- BD ExK DNA-1 Extraction kit (BD cat no: 442818)
- BD MMK (SPC) mastermix (BD cat no: 442829)
- BD MAX PCR Cartridges (BD cat no: 437519)
- Dried snap-ins b-CAP (Biolegio cat no: BDT 14001)
- Vortex Mixer
- Micropipettes
- Pipette tips with filters
- Disposable gloves
- Lab. coat

### 1.2 Run settings

The assay is run on the BD MAX with use of the BD MMK(SPC) in combination with the ExK DNA-1 kit for extraction.

Create a full process assay in the test editor named “b-CAP” using the following channel settings:

Run > Test Editor > Edit > Channel Settings

Test Name: bCAP Extraction Type: ExK DNA-1 (Urine) Master Mix Format: Type 1: BD MMK or MMK (SPC) and Dried Primers and Probes

Channel Settings

Channel	Wavelength	Alias	PCR Gain	Threshold (EP Min)	Ct. Min	Ct. Max	Melt Gain
Channel	475/520	Cpn	- 40 +	- 100 +	- 0 +	- 0 +	- 40 +
	530/565	Lpn	- 60 +	- 150 +	- 0 +	- 0 +	- 60 +
	585/630	Cps	- 40 +	- 100 +	- 0 +	- 0 +	- 40 +
	630/665	Mpn	- 40 +	- 100 +	- 0 +	- 0 +	- 40 +
	680/715	SPC	- 40 +	- 100 +	- 0 +	- 0 +	- 40 +

Color Compensation

Excitation Channel	Wavelength	475/520	530/565	585/630	630/665	680/715
Excitation Channel	475/520	--	- 0 +	- 0 +	- 0 +	- 0 +
	530/565	- 3 +	--	- 0 +	- 0 +	- 0 +
	585/630	- 0 +	- 0 +	--	- 0 +	- 0 +
	630/665	- 0 +	- 0 +	- 0 +	--	- 0 +
	680/715	- 0 +	- 0 +	- 0 +	- 3.4 +	--

To return melt data, test must have melt gain(s) and at least one melt step.

Save Cancel

Back to Test List

Worklist PCR Only Test Editor Kit Inventory

BD Log Out Unlock Door Start Run Status Results Configuration Reports Maintenance



Edit the test steps using the following settings:

Test Name: bCAP Extraction Type: ExK DNA-1 (Urine) Master Mix Format: Type 1: BD MMK or MMK (SPC) and Dried Primers and Probes

**Test Steps**

Step Name: Denaturation

Profile Type: Hold Cycles: 1

Type	Time (s)	Temp (°C)	Detect
	600	98	<input type="checkbox"/>

Step Name: Amplification

Profile Type: 2 - Temperature Cycles: 45

Type	Time (s)	Temp (°C)	Detect
	10	98	<input type="checkbox"/>
	30	63	<input checked="" type="checkbox"/>

Buttons: Move Step, Add Step

If desired apply the result logic as follows;

Test Name: bCAP Extraction Type: ExK DNA-1 (Urine) Master Mix Format: Type 1: BD MMK or MMK (SPC) and Dried Primers and Probes

**Result Logic Steps**

Target	Wavelength	Alias	Analyze
Cpn	475/520	Cpn	<input checked="" type="checkbox"/>
	530/565	Lpn	<input type="checkbox"/>
	585/630	Cps	<input type="checkbox"/>
	630/665	Mpn	<input type="checkbox"/>
	680/715	SPC	<input checked="" type="checkbox"/>
	Lpn	475/520	Cpn
530/565		Lpn	<input checked="" type="checkbox"/>
585/630		Cps	<input type="checkbox"/>
630/665		Mpn	<input type="checkbox"/>
680/715		SPC	<input checked="" type="checkbox"/>
Cps		475/520	Cpn
	530/565	Lpn	<input type="checkbox"/>
	585/630	Cps	<input checked="" type="checkbox"/>
	630/665	Mpn	<input type="checkbox"/>
	680/715	SPC	<input checked="" type="checkbox"/>
	Mpn	475/520	Cpn
530/565		Lpn	<input type="checkbox"/>
585/630		Cps	<input type="checkbox"/>
630/665		Mpn	<input checked="" type="checkbox"/>
680/715		SPC	<input checked="" type="checkbox"/>

Buttons: Edit Logic, Move, Add

And edit the logic for each target as follows;

**Edit Logic**

Target: Cpn

Result	Cpn	SPC
POS	Valid	Valid
POS	Valid	Invalid
NEG	Invalid	Valid
UNR	Invalid	Invalid

OK



### 1.3 Setting up the experiment

- a. Create a Work List on the BD MAX instrument using the b-CAP assay (created in step 1.2) and label the lanes with appropriate sample names.
- b. Load the prepared Sample Buffer Tubes into their corresponding position in the BD MAX racks.
- c. Load the BD MAX racks with the corresponding Unitized Reagent Strips. Note: Shake the strip to ensure liquid is at the bottom of tubes.
- d. Snap-in the BD Extraction tubes (position 1), MMK(SPC) tubes (position 2) and b-CAP tubes (position 3) into the Reagent Strip.
- e. Load the racks and cartridges into the BD MAX and Start Run.

## 2. Results interpretation

- 2.1 For a run to be valid:
  - No BD MAX System failures.
  - Negative control (optional) has a Cq value of -1 for all channels except 680/715 (SPC).
  - Positive control (optional) has a Cq values for all channels.
- 2.2 Interpretation if run is valid:
  - A Cq value of -1 indicates a negative result.
  - A Cq value of 0 indicates that no Cq value could be obtained. The curve needs to be investigated visually.
  - A Cq value for either of the targets indicates a positive result for the corresponding target.
  - The SPC (channel 680/715) should always give a Cq value. A negative value for the SPC indicates inhibition and therefore this sample should be repeated.
  - All curves need to be visually checked for right interpretation.

#### Disclaimer:

PAMM Laboratories is not responsible for the results of the b-CAP assay on the BD MAX system. Using the “open protocol” (i.e. b-CAP dried snap in tubes with primer and probes, together with the BD MMK mastermix) the laboratory itself is responsible for the validation of the assay.