

LIGHT **DIAGNOSTICS**

Temecula, CA 92590

Bartonella OligoDetect™

Cat. No. 3055i

**FOR *In Vitro* Diagnostic Use;
EXPORT ONLY**



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INTERNATIONAL

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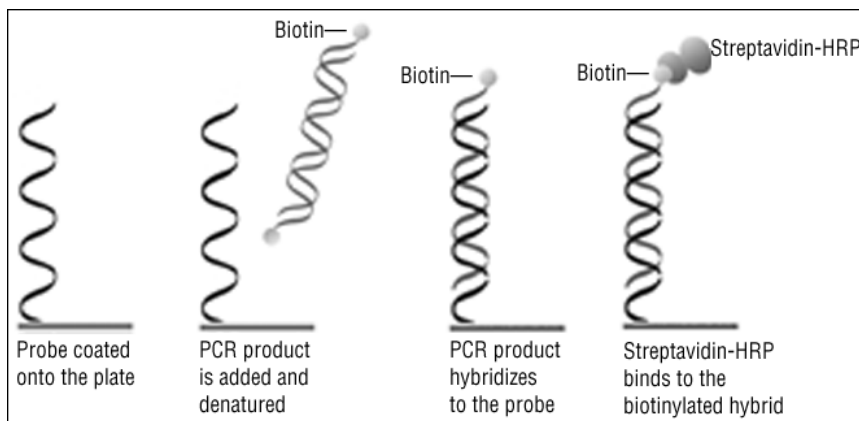
Application

The **Light Diagnostics Bartonella OligoDetect™ Assay** is intended for the qualitative detection of Bartonella DNA generated by an in-house validated *in vitro* nucleic acid amplification of a portion of the 16S Ribosomal RNA(rRNA) gene *B. quintana*, *B. Henselae*, *B. clarridgea*, *B. elizabethae*.

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Principle

The **Light Diagnostics Bartonella OligoDetect™ Assay** is a hybridization capture assay designed to detect amplified nucleic acid specific to the 16S rRNA gene of Bartonella. Amplification reactions are performed using biotinylated primers to generate the required amplification product (amplicon). A portion of the resultant amplicon is added directly to the capture plate and denatured under alkali conditions separating the two DNA strands. The single-stranded amplicon is captured on the plate by hybridization with a specific oligonucleotide probe immobilized to the reaction well. The hybrid complex is then reacted with streptavidin conjugated to horseradish peroxidase (HRP). Unbound conjugate is removed by washing. When TMB substrate is added, a blue color develops. The reaction is stopped by the addition of an acid, changing the color to yellow. This color is quantitated using a microplate reader; the intensity of the color is directly proportional to the amount of amplicon present in the well.



Materials Provided

1. Bartonella OligoDetect™ Plate – (Part No. 5632). One 96-well capture plate for the detection of Bartonella amplicon is provided with removable strips in a vacuum-sealed pouch with desiccant.
2. Bartonella Primer Mix – (Part No. 5631). The lyophilized primer mix contains sense and anti sense primers that map to conserved sequences of the 16S rRNA gene. Reconstitute the lyophilized primer mix with 220 µL of nuclease-free water (15 µM) and store at or below -20°C. Use 2 µL of the primer mix per 100 µL amplification reaction.
3. Bartonella Amplification Positive Control – (Part No. 5634). Lyophilized, amplification positive control (APC) is a DNA molecule containing probe and primer binding sites. Reconstitute the lyophilized APC with 200 µL of nuclease-free water and store at or below -20°C. Use 10 µL of the APC per 100 µL amplification reaction.
4. Bartonella Hybridization Positive Control – (Part No. 5633). Lyophilized, hybridization positive control (HPC) is 5'-biotinylated. Reconstitute the lyophilized HPC with 200 µL of nuclease-free water and store at or below -20°C. Use 10 µL of the HPC per hybridization control reaction.
5. Denaturation Solution – (Part No. 5520). 5 mL of an alkali solution containing a red/orange indicator dye.
6. Hybridization Buffer – (Part No. 5525). 12 mL of buffer containing a blue indicator dye.
7. Wash Buffer, 10X – (Part No. 5702). 100 mL of a concentrated solution containing surfactant and thimerosal. Dilute before use; see Preparation of Reagents.
8. Strep:HRP Conjugate-Ready to Use – (Part No. 5126). 12 mL of a ready to use conjugate solution containing red indicator dye. If a precipitate is present, incubate at 37°C for 10 minutes.
9. Substrate (TMB/E) – (Part No. 5114). 12 mL of a neat solution of 3,3',5,5'-tetramethylbenzidine in a proprietary buffer with enhancer.
10. Stop Solution – (Part No. 5116). 12 mL of an HCl solution.

Materials Required But Not Provided

Reagents:

- Extraction Reagent
- Deionized water (for dilution of the 10X Wash Buffer)
- Nuclease-free water
- Reagents required for nucleic acid amplification

Equipment:

- Thermalcycler
- Spectrophotometric plate reader (450/650 nm) with printer
- 37°C incubator / water bath
- Plate washer (optional)
- Plate cover or mylar sealing tape (optional)

Warnings and Precautions

For In Vitro Diagnostic Use; Export Only

- Some products within this kit contain thimerosal, which is highly toxic by inhalation, contact with skin, or if swallowed. Thimerosal is a possible mutagen and should be handled accordingly.
- Pooling or alteration of any reagent may cause erroneous results.
- Do not substitute reagents from other manufacturers.
- The substrate solution contains TMB, which is highly toxic by inhalation, ingestion, or contact with skin.
- Handle all specimens and materials coming in contact with them as potentially infectious materials. Decontaminate with 0.05% sodium hypochlorite (1:100 dilution of household bleach).
- Do not use reagents beyond expiration date.
- Do not mouth pipette reagents.
- Nucleic acids are subject to degradation by nucleases found in the environment and on human surfaces. Clean and cover work surfaces with disposable coverings and wear powder free gloves during procedure.
- Use pipetting techniques that will deliver correct volumes of reagents in all steps. Repeating pipettors should be primed and voided of all air bubbles.
- Inadequate wash procedures may result in erroneous results.

- With nucleic acid amplification, the potential for carry-over contamination is high. Work in a unidirectional flow from workstation to workstation and always use dedicated equipment and aerosol-barrier pipette tips.
- Incubation times or temperatures other than those specified, may give erroneous results. Any such change must be validated by the user.

Stability and Storage

When stored at 2-8°C, the Light Diagnostics **Bartonella OligoDetect™** is stable up to the expiration date printed on the kit label. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the kit expiration date.

Upon reconstitution, the oligonucleotides can be stored at or below -20°C in low use aliquots to avoid excessive freeze/thaw cycling.

Controls

A **Bartonella Amplification Positive Control** (APC) and an **Bartonella Hybridization Positive Control** (HPC) have been provided. Replicate testing of controls is recommended.

The **APC** is used as a positive control for the amplification reaction. Once amplified, the **APC** can be visualized by electrophoresis as a 100mer double-stranded product. Nuclease-free water or specimen buffer can be used as an amplification negative control (ANC). Use 10 µL of **APC** or ANC for 100 µL amplification reactions.

For verification of the hybridization reactions, assay 10 µL of the **HPC** as a positive control and 10 µL of nuclease-free water as a negative control.

Amplification Procedure

The intended use of this product is to assay amplified nucleic acids produced by various amplification systems, including Polymerase Chain Reaction (PCR), Ligation Chain Reaction (LCR) and Transcription-Mediated Amplification (TMA). This assay has been configured, but is not limited to, the detection of amplified nucleic acids produced by PCR. Consult *PCR Protocols: A Guide to Methods and Applications* (Innis, M., et al, 1990) for specific recommendations.

The PCR reaction components should be prechilled and mixed on ice to minimize nonspecific PCR priming. Hot start PCR is recommended to reduce the formation of primer-dimers and false positive results.

The following protocol is being offered only as a guideline for PCR. To minimize carry-over contamination, it is recommended that uracil-N-glycosylase be included in the PCR procedure. Other validated protocols currently in use for PCR are acceptable.

PCR:

For each PCR reaction prepare the Master Mix as follows (multiply the volumes by the number of reactions, plus one additional volume).

PCR Reagents	100 μL Reaction	Final Conc.
10X PCR Reaction Buffer	10 μ L	1X
10 mM dGTP	2 μ L	200 μ M
10 mM dATP	2 μ L	200 μ M
10 mM dTTP or 20 mM dUTP	2 μ L	200 μ M
10 mM dCTP	2 μ L	200 μ M
25 mM MgCl ₂	4-16 μ L	1mM-4mM
15 μ M Primer Mix	2 μ L	0.3 μ M
Taq Polymerase	0.5 μ L	2.5 U
QS with nuclease-free water		
Sample/Control	10 μ L	
Total reaction volume	100 μ L	

1. Add 90 µL of the Master Mix to the appropriate PCR reaction tubes.
2. Add 10 µL of the negative control to the appropriate PCR reaction tube.
3. Add 10µL of the sample or **APC** to the appropriate PCR reaction tube. Add oil overlay, if necessary, and cap tightly.
4. Place tubes in the Thermocycler and run the following profile:

initial hold:	94°C for 5 minutes
35 cycles of:	94°C for 1 minute 55°C for 30 seconds 72°C for 1 minute
followed by:	72°C for 8 minutes 4°C hold.

Bartonella OligoDetect™ Assay Procedure

- Allow the kit components to equilibrate to room temperature prior to use.
- It is recommended that each reaction be tested in replicate.
- Assay 10 µL of the **HPC** as a positive control and 10 µL of deionized water as a negative control.

Preparation of Reagents

- **All solutions should be completely clear and homogeneous before use.** If necessary warm the **Hybridization Solution** and **Strep:HRP Conjugate** to 37°C then mix until the solution is clear.
- Reconstitute the **Bartonella APC** and **Bartonella HPC** with 200 µL of nuclease-free water and mix well. Reconstituted oligonucleotides can be stored at 2-8°C for up to 1 week. For longer storage, maintain the DNA controls at or below -20°C in low use aliquots to avoid excessive freeze/thaw cycling.
- In a template-free environment, reconstitute the **Bartonella Primer Mix** with 220 µL of nuclease-free water and mix well. The reconstituted primers can be stored at 2-8° C for up to 1 week. For longer storage, maintain the oligonucleotides at or below -20° C in low use aliquots to avoid excessive freeze/thaw cycling.
- Dilute the 10X **Wash Buffer** 10-fold with deionized water (9 parts water to 1 part 10X **Wash Buffer**).

Assay Procedure

The following protocol allows the researcher to denature the amplification product directly in the assay wells. Up to 10 μL of amplicon can be added directly to the assay wells. The **Denaturation** and **Hybridization Buffers** are best added sequentially to the strips with a multichannel pipettor according to the following protocol:

1. Add 10 μL of the amplification product or control to the designated well.
2. Add 5 μL of **Denaturation Solution** to each well and tap the sides of the plate to mix. This addition is most conveniently performed using a multi-channel pipettor, changing the tips between rows to avoid sample contamination. The reactants in the plate will be an orange-yellow color.
3. Incubate for 10 minutes at room temperature.
4. Add 85 μL of **Hybridization Buffer** and tap the sides of the plate to mix. The reaction color will instantly become green with the addition of the **Hybridization Buffer**.
5. Incubate the plate at 37°C for 30 minutes. The plates can be placed in an incubator or floated in a water bath if desired, but should be sealed with mylar sealing tape or plate cover.
6. Wash the plate 3 times with a minimum of 250 μL 1X Wash Buffer per well and blot dry onto a paper towel.
7. Add 100 μL of **Strep:HRP Conjugate** to each well and incubate for 30 minutes at 37°C.
8. Aspirate the **Strep:HRP Conjugate** and wash the plate 3 times as directed in Step 6.
9. Add 100 μL **Substrate** (TMB/E) to each well and incubate at 37°C for 10 minutes.
10. Add 100 μL of **Stop Solution**.
11. **Immediately** (< 5 minutes) determine absorbance values using a spectrophotometric plate reader set at λ_{450} (or dual $\lambda_{450/650\text{nm}}$, if possible). Absorbance values will decrease over time.

Interpretation of Results

It is important to note that amplification procedures and conditions will vary from laboratory to laboratory. It is, therefore, necessary to establish a cut-off value using those specific parameters. **The following interpretation of results is offered only as a guideline.**

Agarose Gel Electrophoresis:

The APC will yield a biotinylated product 100 base pairs (bp) in length when visualized by agarose gel electrophoresis. Bartonella positive samples will yield a 296 bp product.

Bartonella OligoDetect™:

Subtract the averaged absorbance value of the substrate blanks from the mean value of the samples.

The positive (APC) and negative (ANC) controls should fall within the values in the table below:

Positive	$A_{450\text{nm}} \geq 0.200$
Negative	$A_{450\text{nm}} \leq 0.100$
Equivocal	$0.100 < A_{450\text{nm}} < 0.200$

Controls with equivocal results should be retested using the **Bartonella OligoDetect™ Assay**. If equivocal results are again obtained, a second amplification should be performed.

Determine the cut-off point for positive and negative samples by averaging the OD's of duplicate or triplicate negative controls (X_n). Add the average (\bar{X}_n) to 2 standard deviations to obtain the negative cut-off point. Add the average (\bar{X}_n) to 3 standard deviations (SD) to obtain the positive cut-off point. Samples with OD values below the negative cut-off value are negative and samples above the cut-off point are positive. Samples with OD values between the negative and positive cut-off point are equivocal.

Positive	$A_{450\text{nm}} \geq \bar{x}_n + 3\text{SD}$
Negative	$A_{450\text{nm}} \leq \bar{x}_n + 2\text{SD}$
Equivocal	$\bar{x}_n + 2\text{SD} < A_{450\text{nm}} < \bar{x}_n + 3\text{SD}$

Samples with equivocal results should be retested using the **Bartonella OligoDetect™ Assay**. If equivocal results are again obtained, a second amplification should be performed.

The standard deviation (SD) is determined as follows:

$$SD = \sqrt{\frac{\sum (x_n - \bar{x}_n)^2}{n}}$$

where x_n = OD of negative
 n = number of negative determinations
 \bar{x}_n = the average OD at 450_{nm}

Limitations

1. Sample preparation and amplification procedures must be validated to ensure consistency and accuracy of results. It is strongly recommended that the NCCLS Guidelines MM3-A *Molecular Diagnostic Methods for Infectious Diseases* be used as a guideline for quality assurance.
2. Less than 1.5 mM MgCl₂ in the PCR reaction mix may cause a loss of signal intensity, while more than 3 mM will result in a loss of specificity.
3. Less than 2.0 Units of Taq and more than 4.0 Units may give less than optimal results in the detection assay
4. Increasing either the hybridization time to 2 hours or the temperature to 45°C may enhance the signal.

Warranty

These products are warranted to perform as described in their labeling and in *Light Diagnostics* literature when used in accordance with their instructions. THERE ARE NO WARRANTIES WHICH EXTEND BEYOND THIS EXPRESSED WARRANTY AND LIGHT DIAGNOSTICS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. *Light Diagnostics*' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of *Light Diagnostics*, to repair or replace the products. In no event shall *Light Diagnostics* be liable for any proximate, incidental or consequential damages in connection with the products.

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The PCR process is covered by US Patents 4,683,195 and 4,683,202 and foreign equivalents issued to Hoffman-La Roche.

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