AdvanDx

Gram-Negative *Quick*FISH® BC

Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Culture Identification Kit

CE

Σ 25

IVD

REF QFGNRBC1-25

Intended Use

Gram-Negative *Quick*FISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Escherichia coli* and/or *Pseudomonas aeruginosa* and/or *Klebsiella pneumoniae* on smears from positive blood cultures containing Gram-negative bacilli on Gram stain.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.

The Gram-Negative *Quick*FISH BC assay is indicated for use as an aid in the diagnosis of *E. coli,* and/or *K. pneumoniae,* and/or *P. aeruginosa* bacteremia.

IVD For *in vitro* diagnostic use.

Summary and Explanation

E. coli, P. aeruginosa, and K. pneumoniae are recognized as causes of both community and hospital acquired bacteremia.

The identification of *E. coli, P. aeruginosa, and K. pneumoniae* in blood cultures is routinely based on presumptive identification as gram-negative bacilli (GNB) followed by final identification after subculture and biochemical analysis (1).

Gram-Negative *Quick*FISH is a multicolor fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to specific ribosomal RNA sequences of *E. coli, P. aeruginosa* and *K. pneumoniae* (including the three subspecies: *pneumoniae, ozaenae* and *rhinoscleromatis*).

The test provides rapid identification of *E. coli, P. aeruginosa, and K. pneumoniae* on smears made from positive blood cultures.

Principle of the Procedure

A mixture of fluorescein-labeled *E. coli* specific PNA probe, tetramethylrhodamine labeled *P. aeruginosa* specific PNA probe and fluorescein and tetramethylrhodamine labeled *K. pneumoniae* specific PNA probe are added to a smear prepared from a positive blood culture. Hybridization is performed at 55 ± 1 °C for 15 min. and the smear is examined by fluorescence microscopy.

Reagents

Gram-Negative *Quick*FISH BC FISH is comprised of the following kit components:

Gram-Negative PNA Blue	**Gram-Negative PNA Blue** 0.85 mL PNA probes in hybridization solution. Contains 15% formamide.
Gram-Negative PNA Yellow	**Gram-Negative PNA Yellow** 0.85 mL PNA probes in hybridization solution. Contains 15% formamide.

Precautions

IVD For *in vitro* diagnostic use.

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

For professional use only, by personnel trained in laboratory techniques and experienced in fluorescence microscopy.

Safety Precautions

Gram-Negative PNA Blue Gram-Negative PNA Yellow	 Danger Contains 15% Formamide	May cause harm to the unborn child. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
*Quick*Fix-1	Contains 24% ethanol	Harmful to aquatic life with long lasting effects. Safety Data Sheet is available upon request. Available in the *Quick*FISH Fixation Kit.
*Quick*Fix-2	 Danger Contains 97% methanol	Highly flammable liquid and vapor. Toxic if swallowed. Tonic in contact with skin. Toxic if inhaled. Causes damage to the central nervous system. Safety Data Sheet is available upon request. Available in the *Quick*FISH Fixation Kit.

Establish precautions against microbiological hazards.

Do not eat, drink, smoke, apply cosmetics, store or prepare foods within the designated work area.

Dispose of reagents in accordance with federal, state, and local regulations.

Technical Precautions

Reagents must not be used after the expiration dates printed on the labels.

Reagents are provided at fixed concentrations. Assay performance may be affected if the reagents are modified in any way or are not stored under the recommended conditions as detailed in “Storage of Kit Components”.

Avoid microbial contamination of reagents.

Avoid any cross-contamination of samples and reagents, as this may give rise to erroneous results.

Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.

Be sure to use a new pipette tip and inoculating needle for each sample.

Do not use microscope filters other than the AdvanDx Microscope Filters listed in the section for **Materials Required and Available from AdvanDx**.

Do not use microscope slides other than *Quick*FISH Slides (CS012).

It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.

It is important that the microscope is functioning properly. Make sure that the microscope bulb is correctly adjusted and has not aged beyond its specified lifetime.

Light sources other than high pressure mercury vapor arc lamps or modified mercury vapor arc lamps (metal halide) are generally not equivalent in spectral output and intensity and are not recommended for use. Before reporting results with any light source, be sure the Positive Control well displays three distinct fluorescent colors: green, red and yellow.

Storage and Preparation of Kit Components

To ensure optimal kit performance, it is important that kit components are stored according to the following instructions:

Store kit components at 2-8 °C. Store bottles upright and tighten caps after use. Reagents are supplied ready for use.

*Quick*FISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8 °C. Slides must be used immediately after breaking pouch seal. Do not use slides after the expiration date.

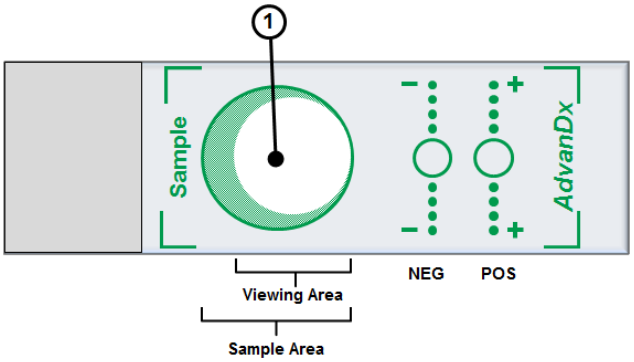
Specimen Collection and Preparation

Preparation of Gram-Negative *Quick*FISH Smears

Gram-Negative *Quick*FISH BC is not compatible with blood culture media containing charcoal or VersaTREK REDOX 2 blood culture bottles.

- Follow the blood culture system manufacturer's instructions to properly mix the blood culture bottle before smear preparation.
- Place slide on SlideStation at 55 ± 1 °C. When running multiple samples, ensure slides do not come in contact with each other to avoid contamination.
- Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.
- Add 1 or more drops of blood culture sample into a secondary vessel (e.g., microcentrifuge tube).
    - For bottles containing resin beads – Add 10 or more drops of sample to an AdvanDx Filter vial. Do not exceed fill line. Insert the filter plunger into the vial and push all the way down to remove the resin beads.
    - Remove cap of AdvanDx Filter Vial to access sample for smear preparation.
- Ensure the blood culture sample is well mixed, using the AdvanDx 10 µL Pipette, transfer 10 µL of sample to the center of the sample area of a *Quick*FISH slide. Refer to reference ① in the *Quick*FISH Slide Diagram.
- Immediately place one drop of *Quick*Fix-1 onto the sample and spread evenly throughout sample area with a plastic inoculating needle. Avoid air bubbles.
- Allow the smear to dry (1-3 minutes). Smear must be visibly dry.
- Add two drops of *Quick*Fix-2 to the center of the sample area. Refer to reference ① in the *Quick*FISH Slide Diagram.
- Allow the smear to dry (~1 minute). Smear must be visibly dry.
- Fixed *Quick*FISH smears may be left on the slide warmer at 55 ± 1 °C for up to 5 minutes. Prepared smears which are not used within 5 minutes can be kept at room temperature for 1 hour prior to testing or may be stored at 2-8 °C for up to 1 day before testing.

***Quick*FISH Slide Diagram**



Test Procedure

Material Provided

Gram-Negative *Quick*FISH BC QFGNRBC1-25

Each kit contains sufficient material for 25 tests. Reagents are supplied ready for use. The expiration date of the kit is as indicated on the outer box label.

Material Required and Available from AdvanDx

Large Coverslips	50 x 24 mm No. 1 Glass coverslip	AC027
AdvanDx Microscope Filter	Dual Band Filter for use with high pressure mercury vapor arc lamp light sources or equivalent	AC007
AdvanDx Metal Halide Filter	Dual Band Filter for use with modified mercury vapor arc lamps (metal halide)	AC033
AdvanDx SlideStation-10	Slide warmer (55 ± 1°C)	AC028
***Quick*FISH Coverslip Mixing Station**	Holds up to 3 coverslips for mixing Gram-negative PNA Yellow & Blue	AC030
AdvanDx 10 µL Pipette	10 µL fixed volume pipette	AC029
***Quick*FISH Slide**	*Quick*FISH slide with controls*	CS012
***Quick*Fix-1**	Primary fixation solution*	CP0169
***Quick*Fix-2**	Secondary fixation solution*	CP0170
AdvanDx Filter Vials	Sample filtration device	AC008

* *Quick*FISH Slide, *Quick*Fix-1, and *Quick*Fix-2 are available in the *Quick*FISH Fixation Kit.

Material Required but Not Provided

- Fluorescence microscope equipped with a 60x or 100x oil objective and a high pressure mercury vapor arc lamp, modified mercury vapor arc lamp (metal halide) or light source with equivalent spectral output.
- Immersion oil. Must comply with the microscope objective and be non-fluorescent.
- Pipette tips
- Plastic inoculating needles.

Assay Procedure:

*Quick*FISH smears should be tested immediately following fixation; however, if smears were stored at 2-8 °C or room temperature they must be placed on the slide warmer for approximately 5 minutes at 55 ± 1 °C before adding the hybridization reagents.

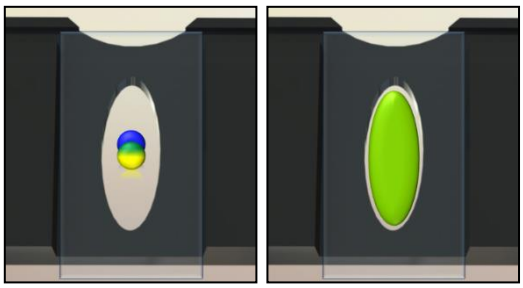
It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.

Use the digital display and the surface thermometer (provided) to verify the SlideStation-10 temperature is 55 ± 1 °C.

Hybridization

- Place a coverslip into one of the *Quick*FISH Coverslip Mixing Station slots. Refer to Diagram #1.
- Invert and hold each bottle and allow a drop to form in the dropper tip before squeezing the bottle to avoid formation of foam in the hybridization mixture.
- Add one drop of Gram-Negative PNA Blue to the center of the coverslip. Note: the ovoid cutout of the *Quick*FISH Coverslip Mixing Station slot denotes the center of the coverslip. Place one drop of Gram-Negative PNA Yellow directly on top of the first drop. Avoid air bubbles. Refer to Diagram #1.
- Thoroughly mix PNA Blue and PNA Yellow together using a plastic inoculating needle until they produce a uniform green color, or no identifiable blue or yellow color remains. Spread lengthwise in order to fill the ovoid template. Refer to Diagram #2.

Diagram #1 **Diagram #2**



- Flip coverslip and apply to slide aligning the edges with the printed border markers on the slide. The coverslip must be placed within the markers. If the coverslip is placed on the white frosted area, the assay may fail due to insufficient flow of reagents.
- Incubate for 15 - 20 min. at 55 ± 1 °C.
- Note: Avoid cross contamination of bottles. Replace dropper caps on appropriate bottles.
- Examine slides as described below

Do not expose the slides to direct sunlight or strong light sources as this may lead to fluorescence bleaching.

Quality Control

Quality control for fluorescent testing should be performed each time testing is performed.

Control material should be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Use *Quick*FISH Slides with Controls (CS012).

*Quick*FISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8 °C. Slides must be used immediately after breaking pouch seal. Do not use slides after expiration date.

The Positive Control will display multiple fluorescent green, red and yellow bacilli. The Negative Control will not contain fluorescent cells. Positive (POS, +) and Negative (NEG, -) control wells contain representative organisms for all AdvanDx *Quick*FISH BC kits. Control organisms for other kits may be weakly visible (non-fluorescent) in both the Positive and Negative Control wells.

Cell morphology may vary between samples and controls due to natural variations.

If the Positive and Negative Controls do not perform in accordance with the Interpretation of Results below, results are invalid and patient results should not be reported.

Locating Controls:

Align the center of the microscope objective with the dots of the POS (+) well on the *Quick*FISH Slide (See *Quick*FISH Slide Diagram). Move the slide stage forward or backward until the green outline of the well appears in the field of view. Use the fine focus knob to focus on the green well outline (this is the correct focal plane for reading the slide). Move the objective into the central region of the POS control to view. To view the NEG control, move the objective laterally into the center of the NEG well. Continue moving laterally to find the viewing area of the sample well.

Procedural Notes

Major Blood Culture Systems and Bottle Type Compatibility:

The *Quick*FISH platform is compatible with commercially available continuously monitoring blood culture systems and bottle types except bottle types supplemented with charcoal and the VersaTREK REDOX 2 Anaerobic bottle. The bottle types tested were:

Clinically:

BacT/ALERT (SA, SN), BACTEC (Lytic/10 Anaerobic/F, Plus Aerobic/F, Standard/10 Aerobic/F)

Analytically:

VersaTREK REDOX 1 Aerobic, BACTEC (Plus Anaerobic/F, Standard/10 Aerobic/F, Standard/10 Anaerobic/F, Peds Plus/F). The clinical performance of these blood culture bottle types with the Gram-Negative *Quick*FISH BC has not been established.

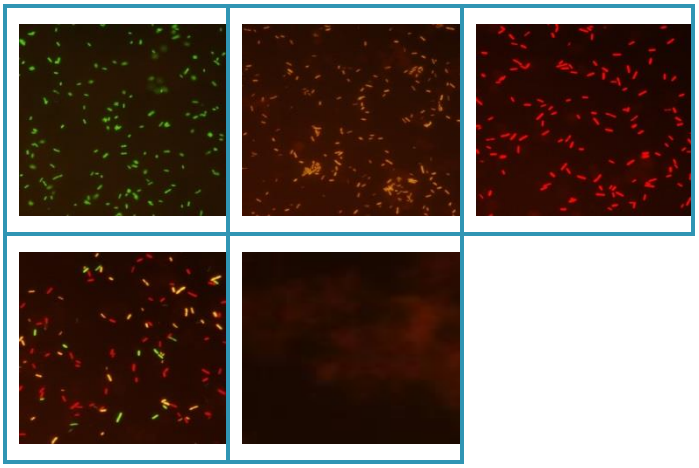
Temperature Control:

It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.

Interpretation of Results

Read slides within 2 hours after hybridization.

Examine slides using a fluorescence microscope equipped with a 60x or 100x oil objective and either the AdvanDx Microscope Filter or AdvanDx Metal Halide Filter. The smear background appears reddish in color. *E. coli* is identified as multiple bright green fluorescent bacilli in multiple fields of view, whereas *P. aeruginosa* is identified as multiple bright red fluorescent bacilli in multiple fields of view and *K*. *pneumoniae* is identified as multiple bright yellow fluorescent bacilli in multiple fields of view. Gram-Negative bacilli that are not *E. coli, K. pneumoniae,* or *P. aeruginosa* appear non-fluorescent. Floating organisms or debris should not be interpreted or confused with positive organisms.



Representative examples of green-positive *E. coli* (top-left); yellow-positive *K. pneumoniae* (top-middle); red-positive *P. aeruginosa* (top-right); mixture of green-positive *E. coli*, yellow-positive *K. pneumoniae* and red-positive *P. aeruginosa* (bottom-left). Negative test results with reddish background (bottom-middle).

Troubleshooting

False Positive and/or Negative Control and Sample test results may occur if the AdvanDx Microscope Filters are not used, or by contamination of the specimens.

False Negative Control or Sample test results may occur if AdvanDx *Quick*FISH Slides (CS012) are not used or if the temperature is not accurately controlled during hybridization.

Please refer to the Precautions and Limitations sections in this product insert or contact AdvanDx.

The lid on the SlideStation is not required to be in place for the kit to perform properly.

The assay may be sensitive to small changes in drop volumes of Gram-Negative PNA Blue and Gram-Negative PNA Yellow. If foam is dispensed from bottles, DO NOT USE, discard the coverslip and prepare a new one using fresh hybridization reagents.

Limitations

- Gram-Negative *Quick*FISH BC is not compatible with blood culture media containing charcoal or VersaTREK REDOX 2 blood culture bottles.
- False Green-Positive results will occur with *Shigella spp.* (serogroups A, B, C, and D), *Escherichia albertii* and *Escherichia fergusonii* due to rRNA sequence similarity.
- False Red-Positive results will occur with *Brevundimonas diminuta, Herbaspirillum huttiense, Pseudomonas fulva, Acinetobacter radioresistens* and some strains of *Pseudomonas putida.*
- False Yellow-Positive results will occur with *Escherichia vulneris* and *Klebsiella variicola*.
- Clinical studies were conducted using the BACTEC Plus Aerobic/F, BACTEC Lytic/10 Anaerobic/F, BacT/ALERT SA and SN blood culture bottles. The performance of Gram-Negative *Quick*FISH BC with other blood culture bottle types has not been established.

- BACTEC Plus Anaerobic/F and BACTEC Peds Plus/F bottles were not extensively evaluated during the clinical investigation, and therefore the performance has not been adequately established.
- The performance of VersaTREK REDOX 1 and BACTEC (Standard/10 Aerobic/F, Standard/10 Anaerobic/F) blood culture bottles was evaluated in an internal compatibility study only. Therefore, the performance is unknown.
- False Positive green autofluorescence may occur if a standard FITC filter is used instead of AdvanDx Microscope Filters.
- False negative results may infrequently occur due to mixed growth or due to error in assay technique.
- The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The fluorescence may vary due to the type of microscope employed, the light source, and the level of rRNA in the cells. Each laboratory should establish its own criteria for reading the results using appropriate controls.
- False-Negative *K. pneumoniae* or False-Positive green results may occur, leading to misidentification of *K. pneumoniae* as *E. coli* if light sources other than high pressure mercury vapor arc lamps and modified mercury vapor arc lamps (metal halide) are used.
- Isolation on solid media is needed to differentiate mixed growth with other organisms and to identify positive blood cultures yielding a negative FISH result.
- The product has not been validated with specimens other than blood cultures.

Expected Results

The clinical study population of Gram-Negative-positive blood culture bottles was derived from 5 healthcare centers in the United States and included 306 blood cultures from 263 patients and 43 spiked samples. The *E. coli, P. aeruginosa* and *K. pneumoniae* positive result rates ranged from 23-38%, 0-12%, and 19-33% respectively. Other gram-negative organisms were identified in 25-48% of the samples.

The rates presented are a percentage of unique patient blood cultures (multiple samples from the same patient and spiked samples were not included) as identified by routine methods as a percent of the total number of all species identified in the studies.

Rates of positive and negative species results obtained with Gram-Negative *Quick*FISH BC may vary depending on institution and patient population (2).

Performance Characteristics

The clinical study population of Gram-Negative-positive blood culture bottles was derived from 5 health care centers in the United States and included 306 blood cultures from 263 patients and 43 spiked samples. The sensitivities of Gram-Negative *Quick*FISH BC against routine laboratory methods are 96.8% (91/94) for *E. coli*, 98.1% (52/53) for *P. aeruginosa*, and 100% (60/60) for *K. pneumoniae,* and the specificity is 99.0% (99/100) from positive blood culture bottles containing gram-negative bacilli.

Clinical Performance Data for Gram-Negative *Quick*FISH BC vs. Routine Identifications on GNB-positive Blood Culture Bottles

Gram-Negative *Quick*FISH	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	Other
E. coli	91[1,3]	0	0	0
P. aeruginosa	0	52[2]	0	1
K. pneumoniae	1	0	60[3]	0
Negative	2	1	0	99
N=307[3]	**Positive Percent Agreement** 96.8% (91/94) 95% CI (91.0-98.9)	**Positive Percent Agreement** 98.1% (52/53) 95% CI (90.1-99.7)	**Positive Percent Agreement** 100% (60/60) 95% CI (94.0-100)	**Negative Percent Agreement** 99.0% (99/100) 95%CI (94.6-99.8)

[1]Includes 9 blood cultures spiked with clinical strains of *E. coli*
[2]Includes 34 blood cultures spiked with clinical strains of *P. aeruginosa.*
[3]Includes 1 mixed culture of *E. coli* and *K. pneumoniae*

Bottles were stored at room temperature after Gram stain and prior to *Quick*FISH testing. Testing was performed within 2 hours 17% of the time, within 4 hours 32% of the time, and within 8 hours 54% of the time. Between 8 and 48 hours after Gram staining, 45% of the testing was completed and 1% in greater than 48 hours.

Limit of Detection

The analytical sensitivity of Gram-Negative *Quick*FISH BC as measured as the detection limit of the assay for *E. coli, P. aeruginosa,* and *K. pneumoniae* was determined to be approximately $2.3x10^5$, $4.0x10^5$ and $4.5x10^5$ CFU/mL, respectively by serial dilutions. This is consistent with the analytical sensitivity of other slide-based staining techniques.

Analytical Specificity and Sensitivity (inclusivity)

Gram-Negative *Quick*FISH BC was tested on 152 reference and clinical laboratory strains including 16 strains of *E. coli*, 21 strains of *P. aeruginosa*, and 12 strains of *K. pneumoniae*. All 16 strains of *E. coli* tested green-positive, all 21 strains of *P. aeruginosa* tested red-positive, and all 12 strains of *K. pneumoniae* tested yellow-positive. In addition to these three target species, testing included 86 strains of other gram-negative bacilli. Seventy-eight of these produced the expected negative results. The following 5 strains produced False Positive green results: *Escherichia albertii, Escherichia fergusonii,* and *Shigella* serogroups A,B C and D. The following 3 strains produced False Positive red results: *Pseudomonas fulva* (2 strains) and *Acinetobacter radioresistens*. Testing also included of 17 strains of other bacteria (11) and yeast (6), all of which tested negative.

Reproducibility

A reproducibility study was performed with Gram-Negative *Quick*FISH BC and the results are presented below by site across 3 days and by day across 3 sites, with 2 operators at each site.

Summary of Reproducibility Results by Site Across 3 Days

	Site 1	Site 2	Site 3	Total
Positive Agreement Green	42/45	45/45	45/45	**97.8% (132/135)**
Positive Agreement Red	42/45	45/45	45/45	**97.8% (132/135)**
Positive Agreement Yellow	45/45	45/45	45/45	**100% (135/135)**
Negative Agreement	42/45	45/45	45/45	**97.8% (132/135)**
Total Agreement	**95.0% (171/180)**	**100% (180/180)**	**100% (180/180)**	**98.3% (531/540)**

Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total
Positive Agreement Green	42/45	45/45	45/45	**97.8% (132/135)**
Positive Agreement Red	45/45	42/45	45/45	**97.8% (132/135)**
Positive Agreement Yellow	45/45	45/45	45/45	**100% (135/135)**
Negative Agreement	45/45	42/45	45/45	**97.8% (132/135)**
Total Agreement	**98.3% (177/180)**	**96.7% (174/180)**	**100% (180/180)**	**98.3% (531/540)**

Bibliography

1. **Baron, E.J.** 1998. Processing and interpretation of blood cultures, chap. 2.3. In: H.D. Isenberg (Ed.) Essential procedures for clinical microbiology, ASM Press, Washington DC.
2. **Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA. 2004.** Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Micro and Antibiol. **3**(7).

Definitions

REF	Product code/catalog number	LOT	Batch code
	Consult the instructions for use		Storage temperature limitations
	Contains sufficient for <n> tests		Health Hazard
	Manufacturer		Skull and Crossbones
EC REP	Authorized Representative		Flame
	Use by		

Technical Advice and Customer Service

For all inquiries, please contact OpGen or your local distributor.

OpGen, Inc.
708 Quince Orchard Rd
Gaithersburg, MD 20878
USA

Tel: +1 301 869 9683
Fax: +1 301 869 9684

techsupport@opgen.com

EC REP

Curetis GmbH
Max-Eyth-Straße 42
71088 Holzgerlingen,
Germany

Tel: +49 7031 49195 10
Fax: +49 7031 49195 19

www.OpGen.com

Produced under license from Boston Probes, Inc.
The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.

30 April 2020

PN2014J-EN
DCR 20-0034

Purchase of this kit licenses its use under patent numbers: US 5,985,563; US 5,888,733; US 6,664,045; US 6,395,474; US 6,357,163; US 5,539,082; US 7,223,833; US 6,361,942; US 7,816,50; EP 862,650; EP 804,456