pcDNA™3.1(+)  
pcDNA™3.1(−)  
Catalog nos. V790-20 and V795-20  

Version K  
10 November 2010  
28-0104
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</tbody>
</table>
Important Information

pcDNA™ Vectors

This manual is supplied with the following products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pcDNA™3.1(+) Vector</td>
<td>V790-20</td>
</tr>
<tr>
<td>pcDNA™3.1(−) Vector</td>
<td>V795-20</td>
</tr>
</tbody>
</table>

Shipping and Storage

Vectors are shipped on wet ice. Upon receipt, store at −20°C.

Contents

The pcDNA™3.1 vector components pcDNA™3.1 are listed below:

<table>
<thead>
<tr>
<th>Item</th>
<th>Concentration</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>pcDNA™3.1 Vector</td>
<td>20 μg at 0.5 μg/μl, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)</td>
<td>40 μl</td>
</tr>
<tr>
<td>pcDNA™3.1(+) or pcDNA™3.1(−)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Plasmid</td>
<td>20 μg at 0.5 μg/μl, in TE buffer, pH 8.0 (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)</td>
<td>40 μl</td>
</tr>
<tr>
<td>pcDNA™3.1/CAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Product Qualification

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.
### Accessory Products

Additional products that may be used with the pcDNA™3.1 vectors are available from Invitrogen. Ordering information is provided below.

<table>
<thead>
<tr>
<th>Product</th>
<th>Amount</th>
<th>Catalog no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Shot® TOP10 Chemically Competent Cells</td>
<td>10 reactions</td>
<td>C4040-10</td>
</tr>
<tr>
<td></td>
<td>20 reactions</td>
<td>C4040-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One Shot® TOP10F’ Chemically Competent Cells</td>
<td>20 reactions</td>
<td>C3030-03</td>
</tr>
<tr>
<td></td>
<td>40 reactions</td>
<td>C3030-06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipofectamine™ 2000</td>
<td>1.5 ml</td>
<td>11668-019</td>
</tr>
<tr>
<td></td>
<td>0.75 ml</td>
<td>11668-027</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geneticin®</td>
<td>1 g</td>
<td>11811-023</td>
</tr>
<tr>
<td></td>
<td>5 g</td>
<td>11811-031</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PureLink™ HQ Mini Plasmid Purification Kit</td>
<td>100 preps</td>
<td>K2100-01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PureLink™ HiPure Plasmid Midiprep Kit</td>
<td>25 preps</td>
<td>K2100-04</td>
</tr>
</tbody>
</table>
Overview

Description

pcDNA™3.1(+) and pcDNA™3.1(−) are 5.4 kb vectors derived from pcDNA™3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (−) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA™3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA™3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA™3.1.
2. Ligate your insert into the appropriate vector and transform into E. coli. Select transformants on LB plates containing 50–100 μg/ml ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.
Cloning into pcDNA™3.1

Introduction

Diagrams are provided on pages 3-4 to help you design a cloning strategy for ligating your gene of interest into pcDNA™3.1. General considerations for cloning and transformation are listed below.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, please refer to *Molecular Cloning: A Laboratory Manual* (Sambrook et al., 1989) or *Current Protocols in Molecular Biology* (Ausubel et al., 1994).

E. coli Strain

Many *E. coli* strains are suitable for the propagation of this vector including TOP10F′, DH5™-T1R, and TOP10. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*recA*) and endonuclease A-deficient (*endA*).

Note

If you wish to express a human gene of interest from pcDNA™3.1, we recommend using an Ultimate™ Human ORF (hORF) Clone available from Invitrogen. For more information about the Ultimate™ hORF Clones available, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 13).

Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintenance of pcDNA™3.1

To propagate and maintain pcDNA™3.1, use 10 ng of vector to transform a *recA*, *endA* *E. coli* strain like TOP10F′, DH5™-T1R, TOP10, or equivalent. Select transformants on LB plates containing 50–100 μg/ml ampicillin. Be sure to prepare a glycerol stock of your plasmid-containing *E. coli* strain for long-term storage (see page 5).

Cloning Considerations

pcDNA™3.1(+) and pcDNA™3.1(−) are non-fusion vectors. Your insert should contain a Kozak translation initiation sequence with an ATG initiation codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). An example of a Kozak consensus sequence is provided below. Other sequences are possible, but the G or A at position –3 and the G at position +4 (shown in bold) illustrates the most commonly occurring sequence with strong consensus. Replacing one of the two bases at these positions provides moderate consensus, while having neither results in weak consensus. The ATG initiation codon is shown underlined.

\[(G/A)NNATGG\]

Your insert must also contain a stop codon for proper termination of your gene. Please note that the *Xba I* site contains an internal stop codon (TCTAGA).

continued on next page
Below is the multiple cloning site for pcDNA™3.1(+). Restriction sites are labeled to indicate the cleavage site. The Xba I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. The complete sequence of pcDNA™3.1(+) is available for downloading from our web site (www.invitrogen.com) or from Technical Support (see page 13). For a map and a description of the features of pcDNA™3.1(+), please refer to the Appendix, pages 10-11.

Please note that there are two BstX I sites in the poly linker.
Cloning into pcDNA™3.1, continued

Multiple Cloning Site of pcDNA™3.1(–)

Below is the multiple cloning site for pcDNA™3.1(–). Restriction sites are labeled to indicate the cleavage site. The Xba I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. The complete sequence of pcDNA™3.1(–) is available for downloading from our web site (www.invitrogen.com) or from Technical Support (see page 13). For a map and a description of the features of pcDNA™3.1(–), please see the Appendix, pages 10-11.

*Please note that there are two BstX I sites in the polylinker.
Cloning into pcDNA™3.1, continued

**E. coli Transformation**

Once you have obtained a clone containing your gene of interest, you may transform the clone into a suitable *E. coli* host (see below). We recommend including a negative control in your experiment to help you evaluate your results.

We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 μg aliquots.

**Preparing a Glycerol Stock**

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at –20°C.

- Streak the original colony out on an LB plate containing 50 μg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 ml of LB containing 50 μg/ml ampicillin.
- Grow the culture to mid-log phase (OD₆₀₀ = 0.5–0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.

Store at –80°C.
Transfection

Introduction
Once you have verified that your gene is cloned in the correct orientation and contains an initiation ATG and a stop codon, you are ready to transfect your cell line of choice. We recommend that you include the positive control vector and a mock transfection (negative control) to evaluate your results.

Plasmid Preparation
Once you have generated your expression clone, you must isolate plasmid DNA for transfection. Plasmid DNA for transfection into eukaryotic cells must be clean and free contamination with from phenol and sodium chloride. Contaminants will kill the cells, and salt will interfere with lipid complexing, decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01), the PureLink™ HiPure Plasmid Midiprep Kit (Catalog no. K2100-04), or CsCl gradient centrifugation.

Methods of Transfection
For established cell lines (e.g. HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in Current Protocols in Molecular Biology (Ausubel et al., 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler et al., 1977), lipid-mediated (Felgner et al., 1989; Felgner and Ringold, 1989) and electroporation (Chu et al., 1987; Shigekawa and Dower, 1988). For high efficiency transfection in a broad range of mammalian cell lines, we recommend using Lipofectamine™ 2000 Reagent (Catalog no. 11668-027) available from Invitrogen. For more information about Lipofectamine™ 2000 and other transfection reagents, refer to our Web site (www.invitrogen.com) or contact Technical Support (page 13).

Positive Control
pcDNA™3.1/CAT is provided as a positive control vector for mammalian transfection and expression (see page 12) and may be used to optimize transfection conditions for your cell line. The gene encoding chloramphenicol acetyl transferase (CAT) is expressed in mammalian cells under the control of the CMV promoter. A successful transfection will result in CAT expression that can be easily assayed (see below).

Assay for CAT Protein
You may assay for CAT expression by ELISA assay, western blot analysis, fluorometric assay, or radioactive assay (Ausubel et al., 1994; Neumann et al., 1987). If you wish to detect CAT protein using western blot analysis, you may use the Anti-CAT Antiserum (Catalog no. R902-25) available from Invitrogen. Other kits to assay for CAT protein using ELISA assay are available from Roche Molecular Biochemicals (Catalog no. 1 363 727) and Molecular Probes (Catalog no. F-2900).
Creating Stable Cell Lines

Introduction

The pcDNA™3.1(+) and pcDNA™3.1(–) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

To obtain stable transfectants, we recommend that you linearize your pcDNA™3.1 construct before transfection. While linearizing the vector may not improve the efficiency of transfection, it increases the chances that the vector does not integrate in a way that disrupts elements necessary for expression in mammalian cells. To linearize your construct, cut at a unique site that is not located within a critical element or within your gene of interest.

Geneticin®

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn\(^5\), results in detoxification of Geneticin® (Southern and Berg, 1982).

Determining Antibiotic Sensitivity

To successfully generate a stable cell line expressing your protein of interest, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. Test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 6–7 plates. Add the following concentrations of antibiotic to each plate:
   - For Geneticin® selection, test 0, 50, 125, 250, 500, 750, and 1000 \(\mu\)g/ml Geneticin®.

2. Replenish the selective media every 3–4 days, and observe the percentage of surviving cells.

3. Count the number of viable cells at regular intervals to determine the appropriate concentration of antibiotic that prevents growth within 1–3 weeks after addition of the antibiotic.

Geneticin® Selection Guidelines

Once you have determined the appropriate Geneticin® concentration to use for selection, you can generate a stable cell line expressing your pcDNA™3.1 construct. Geneticin® is available separately from Invitrogen (see page vi for ordering information). Use as follows:

1. Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).

2. Use the predetermined concentration of Geneticin® in complete medium.

3. Calculate concentration based on the amount of active drug.

4. Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 3 weeks of growth in selective medium.

continued on next page
Prior to transfection, we recommend that you linearize the pcDNA™3.1(+) vector. Linearizing pcDNA™3.1(+) will decrease the likelihood of the vector integrating into the genome in a way that disrupts the gene of interest or other elements required for expression in mammalian cells. The table below lists unique restriction sites that may be used to linearize your construct prior to transfection. **Other unique restriction sites are possible.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Restriction Site (bp)</th>
<th>Location</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgl II</td>
<td>12</td>
<td>Upstream of CMV promoter</td>
<td>Invitrogen, Catalog no. 15213-028</td>
</tr>
<tr>
<td>Mfe I</td>
<td>161</td>
<td>Upstream of CMV promoter</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>Bst1107 I</td>
<td>3236</td>
<td>End of SV40 polyA</td>
<td>AGS®, Fermentas, Takara, Roche Mol. Biochemicals</td>
</tr>
<tr>
<td>Eam1105 I</td>
<td>4505</td>
<td>Ampicillin gene</td>
<td>AGS®, Fermentas, Takara</td>
</tr>
<tr>
<td>Pvu I</td>
<td>4875</td>
<td>Ampicillin gene</td>
<td>Invitrogen, Catalog no. 25420-019</td>
</tr>
<tr>
<td>Sca I</td>
<td>4985</td>
<td>Ampicillin gene</td>
<td>Invitrogen, Catalog no. 15436-017</td>
</tr>
<tr>
<td>Ssp I</td>
<td>5309</td>
<td>bla promoter</td>
<td>Invitrogen, Catalog no. 15458-011</td>
</tr>
</tbody>
</table>

*Angewandte Gentechnologie Systeme

The table below lists unique restriction sites that may be used to linearize your pcDNA™3.1(−) construct prior to transfection. **Other unique restriction sites are possible.** Be sure that your insert does not contain the restriction enzyme site you wish to use to linearize your vector.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Restriction Site (bp)</th>
<th>Location</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgl II</td>
<td>12</td>
<td>Upstream of CMV promoter</td>
<td>Invitrogen, Catalog no. 15213-028</td>
</tr>
<tr>
<td>Mfe I</td>
<td>161</td>
<td>Upstream of CMV promoter</td>
<td>New England Biolabs</td>
</tr>
<tr>
<td>Bst1107 I</td>
<td>3235</td>
<td>End of SV40 polyA</td>
<td>AGS®, Fermentas, Takara, Roche Mol. Biochemicals</td>
</tr>
<tr>
<td>Eam1105 I</td>
<td>4504</td>
<td>Ampicillin gene</td>
<td>AGS®, Fermentas, Takara</td>
</tr>
<tr>
<td>Pvu I</td>
<td>4874</td>
<td>Ampicillin gene</td>
<td>Invitrogen, Catalog no. 25420-019</td>
</tr>
<tr>
<td>Sca I</td>
<td>4984</td>
<td>Ampicillin gene</td>
<td>Invitrogen, Catalog no. 15436-017</td>
</tr>
<tr>
<td>Ssp I</td>
<td>5308</td>
<td>bla promoter</td>
<td>Invitrogen, Catalog no. 15458-011</td>
</tr>
</tbody>
</table>

*Angewandte Gentechnologie Systeme

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Creating Stable Cell Lines, continued

Selection of Stable Integrants

Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

1. Transfect your mammalian host cell line with your pcDNA™3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA™3.1/CAT plasmid as a positive control.

2. 24 hours after transfection, wash the cells and add fresh medium to the cells.

3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.

4. Feed the cells with selective medium every 3–4 days until Geneticin®-resistant foci can be identified.

5. Pick and expand colonies in 96- or 48-well plates.
Appendix

pcDNA™3.1 Vectors

Map

The figure below summarizes the features of the pcDNA™3.1(+) and pcDNA™3.1(−) vectors. The complete sequences for pcDNA™3.1(+) and pcDNA™3.1(−) are available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Support (see page 13). Details of the multiple cloning sites are shown on page 3 for pcDNA™3.1(+) and page 4 for pcDNA™3.1(−).

Comments for pcDNA3.1 (+)
5428 nucleotides
CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
tcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (bla): bases 4432-6428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

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pcDNA™ 3.1 Vectors, continued

Features: pcDNA™3.1(+) (5428 bp) and pcDNA™3.1(−) (5427 bp) contain the following elements. All features have been functionally tested.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human cytomegalovirus (CMV) immediate-early promoter/ enhancer</td>
<td>Permits efficient, high-level expression of your recombinant protein (Andersson et al., 1989; Boshart et al., 1985; Nelson et al., 1987)</td>
</tr>
<tr>
<td>T7 promoter/priming site</td>
<td>Allows for <em>in vitro</em> transcription in the sense orientation and sequencing through the insert</td>
</tr>
<tr>
<td>Multiple cloning site in forward or reverse orientation</td>
<td>Allows insertion of your gene and facilitates cloning</td>
</tr>
<tr>
<td>Bovine growth hormone (BGH) polyadenylation signal</td>
<td>Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)</td>
</tr>
<tr>
<td>f1 origin</td>
<td>Allows rescue of single-stranded DNA</td>
</tr>
<tr>
<td>SV40 early promoter and origin</td>
<td>Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen</td>
</tr>
<tr>
<td>Neomycin resistance gene</td>
<td>Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)</td>
</tr>
<tr>
<td>SV40 early polyadenylation signal</td>
<td>Efficient transcription termination and polyadenylation of mRNA</td>
</tr>
<tr>
<td>pUC origin</td>
<td>High-copy number replication and growth in <em>E. coli</em></td>
</tr>
<tr>
<td>Ampicillin resistance gene (β-lactamase)</td>
<td>Selection of vector in <em>E. coli</em></td>
</tr>
<tr>
<td>Ampicillin (bla) resistance gene (β-lactamase)</td>
<td>Allows selection of transformants in <em>E. coli</em></td>
</tr>
</tbody>
</table>
pcDNA™3.1/CAT

Description

pcDNA™3.1/CAT is a 6217 bp control vector containing the gene for CAT. It was constructed by digesting pcDNA™3.1(+) with Xho I and Xba I and treating with Klenow. An 800 bp Hind III fragment containing the CAT gene was treated with Klenow and then ligated into pcDNA™3.1(+).

Map

The figure below summarizes the features of the pcDNA™3.1/CAT vector. The complete nucleotide sequence for pcDNA™3.1/CAT is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Support (see page 13).

Comments for pcDNA3.1(+)CAT

- CMV promoter: bases 232-819
- T7 promoter/priming site: bases 863-882
- CAT ORF: bases 1027-1686
- pcDNA3.1/BGH reverse priming site: bases 1811-1828
- BGH polyadenylation sequence: bases 1817-2041
- fl origin: bases 2087-2515
- SV40 early promoter and origin: bases 2520-2883
- Neomycin resistance gene (ORF): bases 2925-3719
- SV40 early polyadenylation sequence: bases 3893-4023
- pUC origin: bases 4406-5076 (complementary strand)
- Ampicillin resistance gene (ORF): bases 5221-6081 (complementary strand)
Technical Support

World Wide Web

Visit the Invitrogen website at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: euretech@invitrogen.com

MSDS
Material Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/msds.

Certificate of Analysis
The Certificate of Analysis (CofA) provides detailed quality control information for each product. CofAs are available on our website at www.invitrogen.com/support, and are searchable by product lot number, which is printed on each box.

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Introduction

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