Inqaba biotec™

Oligos – Frequently Asked Questions

Have other questions not answered below? Please contact

How do I order oligonucleotides?

For your convenience, inqaba biotec™ has provided its customers with an efficient online system to order oligos directly from our customer portal.

For step-by-step instruction manual on how to order please contact us and we will guide you through the process.

Please note that an order will only be released for dispatch once a purchase order number or payment is received.

How long does delivery take?

Please find below tabulated information regarding delivery of oligonucleotides.

Oligo Service	**Overnight Delivery***
Standard Oligos (desalted or cartridge purified)	48 hours (if order received before 9 am)
PAGE Purified Oligos	72 hours
Modified Oligos	72 hours
Probes	72 hours

* Delivery time for oligos passing the quality check and for orders up to 20 oligos.

Products are normally delivered before 11.00 am. All inqaba biotec™ oligonucleotides are shipped lyophilized. Customers outside South Africa will expect an extra charge for effective courier.

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What scales of oligonucleotide synthesis does inqaba biotec™ provide?

Tabulated below are the different synthesis scales we offer with the respective minimum yield.

Synthesis scale (µmole)	Yield (desalted)*
0.01	3 OD
0.05	6 OD
0.20	15 OD
1.00	40 OD

*Mass yield is dependent on length of the oligo when measured with optical density

All desalted custom synthesized oligos are sent lyophilized. Please ensure that you spin the primer tube for a minute to ensure that your primer pellet does not escape when opening your tube to dissolve your primer.

Minimum Guaranteed yield for standard 20mer oligo: The scale of the oligo synthesis describes the amount of the CPG material to start the synthesis. The yield of the final oligo product is measured at 260nm. 1 OD for a standard 20mer oligo corresponds to approximately 5 nmole of the oligo product.

All standard desalted oligos are deprotected and desalted to remove impurities.

What modifications can we add to an oligonucleotide for Conventional PCR?

inqaba biotec™ offers a range of oligonucleotide modifications for both 5' and 3' ends, as well as internal modifications.

Modification	Position	Details
Wobbles	5', 3', Internal	Useful in projects where degenerate oligonucleotides are needed.
Phosphorylation	5'	Facilitates ligation reactions and linker insertions.
Biotin	5' & 3'	Biotin labelled oligos can be detected through Avidin or Streptavidin-enzyme conjugates. They are also used in solid phase capture by Streptavidin coated magnetic beads.
C6 - Amino	5'	6' amino group protects the 5' end of the oligo from 5' exonucleases. Also used in linker systems.
C3/C7 - Amino	3'	3'- amino group protects the oligo 3' end from 3' exonucleases.
Inosine	5', 3', Internal	Used to lower the specificity of oligos based on the ability of inosine to pair with other bases. This feature allows for the detection of distinct but similar DNA sequences in hybridisations or PCR.
Phosphate	3'	3'- phosphate group protects the oligo 3' end from 3' exonucleases.

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What modifications can we add to an oligonucleotide for Real-Time PCR?

inqaba biotec™ not only synthesizes primers for conventional PCR, but also probes for quantitative or real-time PCR. There are three main types of real-time PCR probes; TaqMan probes, Molecular Beacons and dual-oligo FRET probes. inqaba biotec™ synthesizes probes in all three classes. We have most of the 5' fluorescent dyes as well as the 3' quencher dyes. Following are tables of available fluorescent and quencher dyes.

Modification	Position	Absorption (nm)	Emission (nm)
FAM	5'	492	515
TET	5'	521	536
*JOE	5'	527	548
*VIC	5'	528	546
HEX	5'	535	556
CAL Fluor Orange 560	5'	538	559
*Quasar 570	5'	548	566
Cy3	5'	552	565
*TAMRA	5'	555	580
*ROX	5'	575	602
Cy3.5	5'	581	596
*TexasRed	5'	583	603
*CAL Fluor Red 610	5'	590	610
CAL Fluor Red 635	5'	618	637
*Quasar 670	5'	647	667
Cy5	5'	651	674
Cy5.5	5'	675	694
Dabcyl	3'	453	N/A
Fluoroscein	3'	492	515
BHQ 1	3'	534	480 - 580
BHQ2	3'	579	550 - 670

*Dyes not available at inqaba biotec™

Black Hole Quenchers[1] (BHQ)

The Black Hole Quencher (BHQ) dyes are a modern class of highly efficient dark quenchers that prevent fluorescence until a hybridisation event occurs. Adding a specific fluorescently-labelled hybridisation probe as the reporter instead of the DNA binding dye allows for the detection of only specific amplification products. The BHQ family readily permits single tube multiplexing due to the increased variety of reporter dyes that can be effectively quenched with little or no cross talk between reporters.

[1]Black Hole Quencher, BHQ-1, BHQ-2, CAL Fluor Orange 560, CAL Fluor Red 610, CAL Fluor Red 635, Quasar 570, and Quasar 670 are registered trademarks of Biosearch Technologies, Inc., Novato, California, U.S.A. (BTI) Patents are currently pending for the BHQ technology and such BHQ technology is licensed by the manufacturer pursuant to an agreement with BTI and these products are sold exclusively for research and development use only. They may not be used for human or veterinary in vitro or clinical diagnostic purposes and they may not be re-sold, distributed or re-packaged. For information on licensing programs to permit use for human or veterinary in vitro or clinical diagnostic purposes, please contact Biosearch at licensing@biosearchtech.com.

TaqMan[2] Probes

TaqMan PCR is at present, the most frequently used method for real-time PCR. The probes are longer than primers (20-30 bases long) with a Tm value of 10°C higher. They are designed to anneal to an internal region of a PCR product and contain a fluorescent dye on the 5' base and a quenching dye on the 3' base. When irradiated the excited fluorescent dye transfers energy to the quenching dye (FRET). Close proximity of dye and quencher prevents emission while probe is intact. When Taq replicates a template on which the probe is bound, the 5' exonuclease activity of Taq cleaves the oligonucleotide. Cleavage only occurs if the probe hybridizes to the target and the origin of the fluorescence is specific amplification. Specific requirement: No G must be present at the 5' end as a G adjacent to the reporter dye quenches reporter fluorescence even after cleavage.

[2]TaqMan is a registered trademark of Roche Molecular Systems, Inc., Alameda, CA. PCR is a proprietary technology covered by several US patents including US Patent Nos. 4,683,195, 4,683,202 and 4,965,188, and by issued and pending counterparts outside the U.S. (PCR Patents). PCR Patents are owned by Roche Molecular Systems Inc. and rights under the PCR Patents have been obtained by Applied Biosystems in certain fields.

inqaba biotec™ Reporter – Quencher Pairs

5' Reporter Dye	3' Quencher
CAL Fluor Orange 560	BHQ-1
FAM	
CAL Fluor Orange 590 (TAMRA alternative)	
HEX	
TET	
CAL Fluor Red 610 (Texas Red® alternative)	BHQ-2
CAL Fluor Red 635	
Cy3	
Cy3.5	

Molecular Beacons

Molecular Beacons are hairpin-loop shaped single-stranded oligonucleotides consisting of a probe sequence that is homologous to the target sequence flanked by complementary 'arm' sequences which are homologous to one another and not the target sequence. A fluorescent reporter is covalently attached to one arm and a quencher to the other arm. In the absence of the target sequence the fluorescent reporter is held in close proximity to the quencher and any energy from the reporter is transferred to the quencher. When the probe bounds to its target, the greater stability of the probe-target helix forces the stem to unwind, resulting in a

separation of the reporter from the quencher and now fluorescence can be monitored from the reporter molecule.

inqaba biotec™ Reporter – Quencher Pairs

5' Reporter Dye	3' Quencher
CAL Fluor Red 610	Dabcyl
FAM	
HEX	
TET	
CAL Fluor Orange 560	BHQ-1
FAM	
HEX	
TET	
CAL Fluor Red 610	BHQ-2
CAL Fluor Red 635	
Cy3	
Cy3.5	

Dual-Oligo FRET probes

Dual-oligo FRET (Fluorescence Resonance Energy Transfer) Probes rely on the transfer of energy from one fluorescent molecule to another. Two separate sequence specific oligonucleotides are fluorescently labelled. The upstream probe has a donor molecule on the 3’ end and the downstream probe has an acceptor molecule on the 5’ end. Once the probes hybridize, the donor and acceptor fluorescent molecules are in close proximity of one another. This allows for the transfer of energy from the donor to the acceptor fluorophore, which emits a signal of a different wavelength. Either the decrease in the fluorescence of the donor or the increase in the fluorescence of the acceptor can then be detected. Thus, only when both probes are bound is the transfer of fluorescence detectable.

FRET probes allow for melt curve analysis and are useful in genotyping, SNP detection and other mutation detections.

inqaba biotec™ Reporter – Quencher Pairs

Acceptor	Donor
5’ Cy3.5	3’ Fluorescein
Cy5.5	
CAL Fluor Red 635	

What oligonucleotide synthesis purification options are provided by inqaba biotec™?

Desalting

At inqaba biotec™, all oligonucleotides are desalted as standard. This procedure removes residual by-products from synthesis-, cleavage- and de-protection steps but does not remove shorter synthesis products from full-length oligos. Desalting is sufficient for most PCR applications. However, for specific reactions and for oligos >30 nucleotides we recommend an additional purification step; through cartridge or PAGE purification. Delivery time is 48 hours.

Suitable Applications: PCR, Probing, Mobility Shift, Hybridisation and Sequencing.

Reverse phase cartridge purification

Reverse-phase Cartridge Purification is comparable to HPLC purification in the level of purity (80-95%), and often has a higher recovery for the smaller synthesis scales.

The basis of separation is in the difference in hydrophobicity between full length oligos (contain a 5'-DMT group) and truncated sequences (no DMT group). Cartridge purification is not recommended for oligos > 50 bases because the hydrophobicity between the full length-DMT product and non-DMT truncated sequences are reduced as the oligo length increases. A recovery of ca. 60% can be expected from crude oligo samples. Delivery time is 48 hours.

Suitable Applications: Modified oligos for DNA Fingerprinting and qPCR, Cloning and Polymorphism detection.

PAGE purification

PAGE Purification has the highest purity level of up to 99%. Products differing in only one nucleotide can be separated from the full-length product. It is the purification method suggested for oligos longer than 50 nucleotides. A recovery of only 10% can be expected from crude oligo samples due to the relative inefficient extraction of oligos from the gel. Delivery time is 4 working days.

Suitable Applications: Modified oligos, Gene synthesis, Mutagenesis, Primer extension and Antisense experiments.

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What yield of oligonucleotide can we expect to obtain from the oligonucleotide purification methods provided?

The Table below lists the different scales of synthesis along with the corresponding minimum guaranteed yields associated with the different purification methods:

Scales of Synthesis and Min. Guaranteed Yields per Purification Method

Scale of Synthesis	Desalting*	Cartridge Purification*	PAGE Purification*
10 nmole	3 OD	0.5 OD	0.25 OD
50 nmole	6 OD	1 OD	0.5 OD
200 nmole	15 OD	3 OD	1 OD
1'000 nmole	40 OD	12 OD	5 OD

* Minimum Guaranteed Yield

Minimum Guaranteed Yield for standard 20mer oligo: The scale of the oligo synthesis describes the amount of the CPG material to start the synthesis. The yield of the final oligo product is measured at 260 nm. One OD for a standard 20mer oligo corresponds to approximately 5 nmole of the oligo product.

Which oligonucleotide purification method should I choose for my oligo?

In DNA synthesis, each nucleotide is coupled sequentially to the growing chain. However, in each coupling cycle, a small percentage (usually 1% or less) of the oligo chains will not be extended, resulting in a mixture of full length product and truncated sequences.

After the oligo is cleaved from the support and the protecting groups are removed, purification can separate the full-length product from the truncated sequence products. In general, the oligonucleotide purity required for a specific application depends on the potential problems from the presence of failure sequences (n-1, n-2…). For some applications, the presence of shorter oligos will not affect the experimental results. For other applications, it is imperative that only full length oligos be present. The table below lists the type of applications and the types of purifications recommended for these applications.

Some of the more common primer applications and the different purification types which we apply to different primer applications.

Type of application	Desalting	Cartridge	PAGE
Standard PCR (up to 30 bases)	X		
Standard PCR (31 bases -35 bases)		X	
Standard PCR (36 bases -upwards)			X
Real Time PCR incl. Molecular probes, TaqMan probes, FRET probes			X
Degenerate primers		X	
GC clamp primers	X		
Primers for molecular cloning			X
DGGE primers	X		
FISH probes			X

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Can inqaba biotec™ provide gene synthesis services?

Yes, inqaba biotec™ can also custom synthesize genes. All genes are sequence verified and cloned. For more information on this service please contact us.

inqaba biotec™ guarantees support for all oligo requirements.

The inqaba biotec™ team is strongly rooted in the research community. We are aware of your specific needs and it is our goal to provide you with the best service possible. This includes high quality, fast delivery times and competitive prices.

Please do not hesitate to contact us should you have any questions, require solutions or help with a specific project.

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