PentaBase

PRODUCT CATALOGUE

Customised Oligonucleotides and PCR Assays

2024

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PentaBase

Inventors of INA® - Experts in real-time PCR

PentaBase is a Danish science-driven company established in 2007 by CEO Ulf Bech Christensen. Our products and services are based on unique DNA technologies providing innovative and superior PCR workflows. Our mission is to provide PCR-based assays that enable personalised treatment using sensitive, specific and robust monitoring of genetic biomarkers.

Our approach is to continuously innovate and improve our products and services to provide novel or improved PCR-based solutions for the benefit of patients.

We see every patient as a unique individual who deserves to be treated individually. It is pretty simple: We wish to participate in creating a healthier society through personalised detection, prevention, and treatment of diseases, based on precise and timely genetic diagnostics.

We strive to use our expertise in PCR to offer our customers the best service possible and we aim to combine fast delivery of superior quality products with optimal support before, during, and after the purchase.

Version: 2.5
Last revised: November 2023


Hydrol
5'Penta

Intercalating Nucleic Acid - INA®

We supply customized oligonucleotides and PCR assays that are based on our unique and proprietary Intercalating Nucleic Acid (INA®) technology*. Intercalating Nucleic Acids are made by adding Intercalating Pseudo Nucleotide (IPN) modifications to standard DNA oligonucleotides during synthesis. IPNs are hydrophobic nucleoside analogues that are added to the growing oligonculeotide chain without replacing exisiting nucleotides.

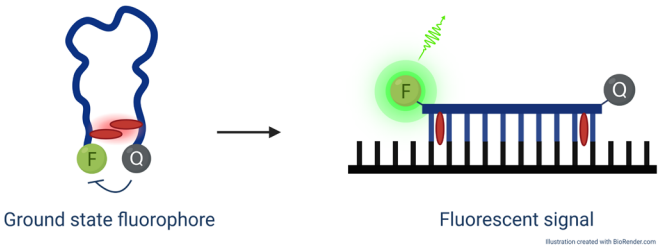
INA® is the only DNA platform technology that works by increasing the π stacking effect of nucleotide base pairs. The result is a much higher affinity and specificity of INA® oligonucleotides towards the target nucleotide sequence compared to standard DNA oligonucleotides. This allows for enhanced discrimination against mismatches. In addition, temperature independet interactions between IPN modifications of non-target bound INA®-based fluorescent probes result in low background fluorescence at all temperatures.

Our EasyBeacon™ and HydrolEasy® probes form the backbone of our flourescence-based assays providing nuclease-resistance, high target specificity and high signal-to-noise ratio. Our SuPrimers™ reduce primer-dimer formation and reduce off-target binding, and our BaseBlockers™ allow for extremely selective amplification of mutated DNA in a wild type DNA background.

At PentaBase A/S we have developed a wide range of INA®-based PCR applications for our customers as well as for our in vitro diagnostic assays for molecular oncology and infectious disease.

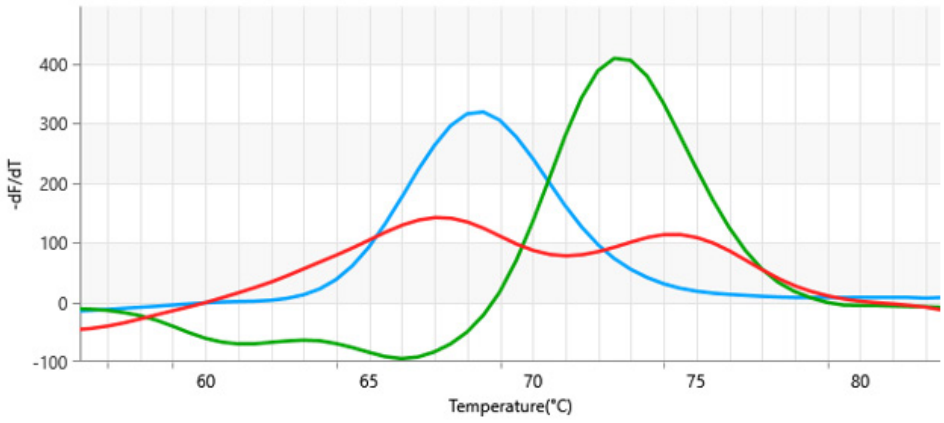
* 1. Christensen UB, Pedersen EB. 2002. https://www.doi.org/10.1093/nar/gkf624
2. Christensen UB, et al. 2004. https://www.doi.org/10.1081/ncn-120027829
3. Nielsen CB, et al. 2004. Doi: https://www.doi.org/10.1021/bc0341932

EasyBeacon™ probes



The perfect probe for end-point melt analyses. The INA® technology used in EasyBeacon™ probes creates a secondary structure in the unbound state that efficiently quenches the signal resulting in a very low background fluorescence without adding a stem sequence. The IPNs in the probes furthermore prevent hydrolysis, making the probes nuclease resistant and therefore intact after amplification.

The high specificity and low background makes it possible to easily differentiate between single nucleotide polymorphisms (SNPs) as well as multiplexing in end-point melting curve analyses.

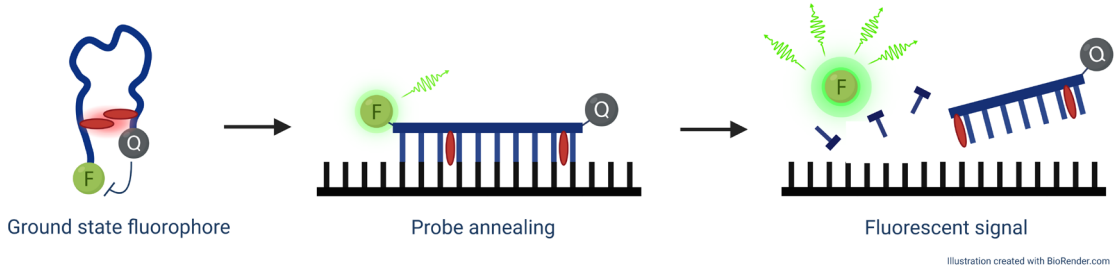


The example shows an EasyBeacon™ assay analysing a human genomic DNA target region with a single nucleotide polymorhism (SNP). A difference in melting temperature of 4°C between the wild type target and the SNP target makes it easy to distinguish between homozygous wild type (blue line), homozygous mutant (green line) and heterozygous (red line).

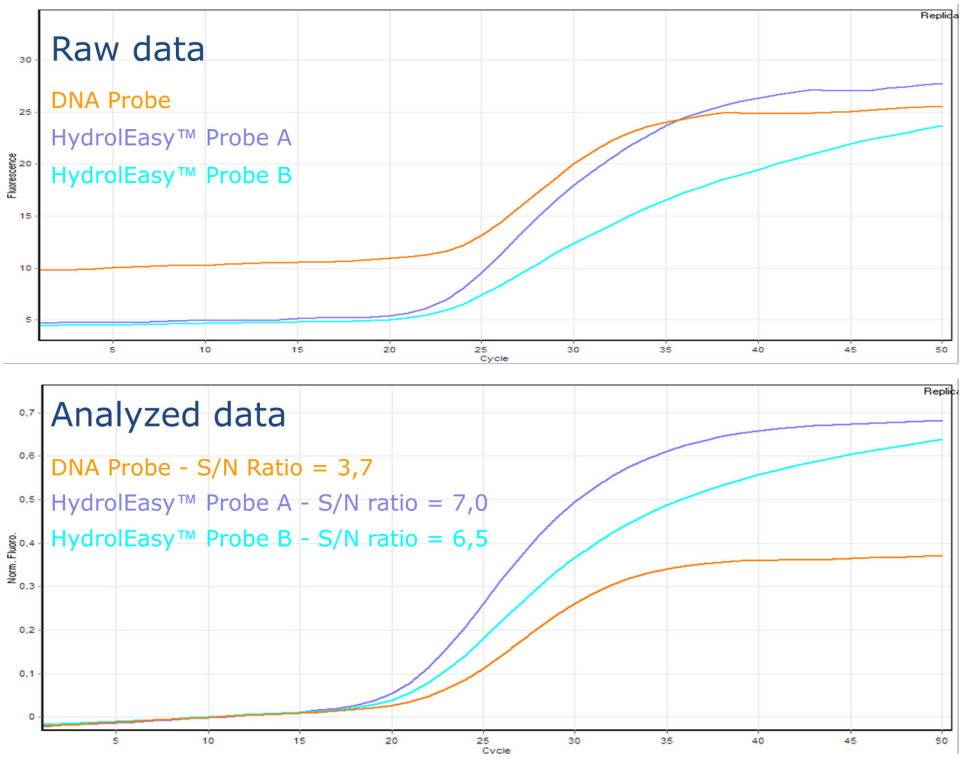
EasyBeacon™ probe advantages

- Ideal for end-point melt analyses.
- Nuclease resistant – intact after amplification.
- Unbound probe is quenched at all temperatures - no need to add a stem
- Ideal for multiplexed genotyping and Hign Resolution Melt (HRM) applications

HydrolEasy® probes



The HydrolEasy® probe is an improved alternative to TaqMan hydrolysis probes. The INA® technology used in HydrolEasy® probes creates a secondary structure in the unbound state that efficiently quenches the signal resulting in a very low background fluorescence and consequently higher signal-to-noise ratio compared to standard hydrolysis probes.



The example shows the raw fluorescence and normalized fluorescence data of two HydrolEasy® probe assays compared to standard hydrolysis probe assay. The lower background fluorescence of the HydrolEasy® probes results in significatly higher signal-to-noise (S/N) ratios.

HydrolEasy® probe advantages

- Unbound probe is quenched at all temperatures
- Enhanced signal-to-noise ratio
- Improved target specificity

Fluorophores & Quenchers

Fluorophore	Excitation	Emission	Quenchers
ATTO 390	390 nm	476 nm	BHQ-1 λ (max)=534nm Range=480-580nm; Dabcyl λ (max)=453nm Range=380-530nm; Tamra λ (max)=544nm Range=470-560nm; Penta GQ/YQ λ (max)=531nm Range=480-580nm
ATTO 425	439 nm	485 nm	
ATTO 465	453 nm	509 nm	
Penta Green	494 nm	518 nm	
6-FAM	494 nm	518 nm	
ATTO 495	498 nm	526 nm	
ATTO 490LS	498 nm	658 nm	
ATTO 488	500 nm	520 nm	
Tide Fluor™ 2 (TF2)	503 nm	525 nm	
6-JOE	520 nm	548 nm	
6-TET	521 nm	536 nm	
CAL Fluor® Gold 540	522 nm	543 nm	
ATTO 532	532 nm	552 nm	
6-HEX	535 nm	556 nm	
CAL Fluor® Orange 560	537 nm	558 nm	
Penta Yellow	538 nm	551 nm	
Quasar® 570	547 nm	570 nm	
CY-3	550 nm	570 nm	BHQ-2 λ (max)=579nm Range=550-650nm; BBQ-650 λ (max)=650nm Range=550-750nm
Penta Dark Yellow	550 nm	570 nm	
ATTO 550	554 nm	576 nm	
Tamra	555 nm	580 nm	
ATTO 565	564 nm	590 nm	
Penta Orange	577 nm	601 nm	
ROX	578 nm	602 nm	
Penta Light Orange	581 nm	594 nm	
CAL Fluor® Red 610	590 nm	610 nm	
California Red™	591 nm	608 nm	
ATTO 590	593 nm	622 nm	
ATTO 594	603 nm	626 nm	
Quasar® 670	644 nm	670 nm	
ATTO 647N	646 nm	664 nm	
CY-5	650 nm	670 nm	
Penta Red	650 nm	670 nm	
Penta Purple	675 nm	694 nm	
Quasar® 705	690 nm	705 nm	
ATTO 700	700 nm	716 nm	
ATTO 725	728 nm	751 nm	

Quencher	Range
BHQ™-1	480-580 nm
BHQ™-2	550-650 nm
BBQ-650™	550-750 nm
Dabcyl	380-530 nm
Tamra	470-560 nm
Penta GQ/YQ	480-580 nm

Quencher	λ (max)
BHQ™-1	534 nm
BHQ™-2	579 nm
BBQ-650™	650 nm
Dabcyl	453 nm
Tamra	544 nm
Penta GQ/YQ	531 nm

BaseBlocker™

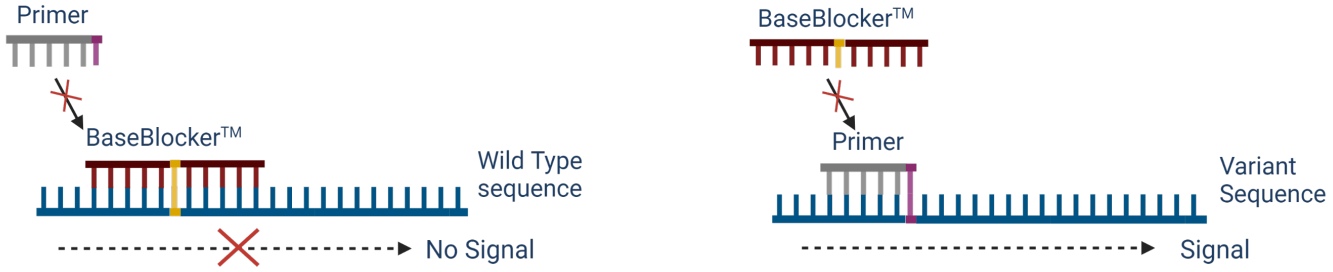
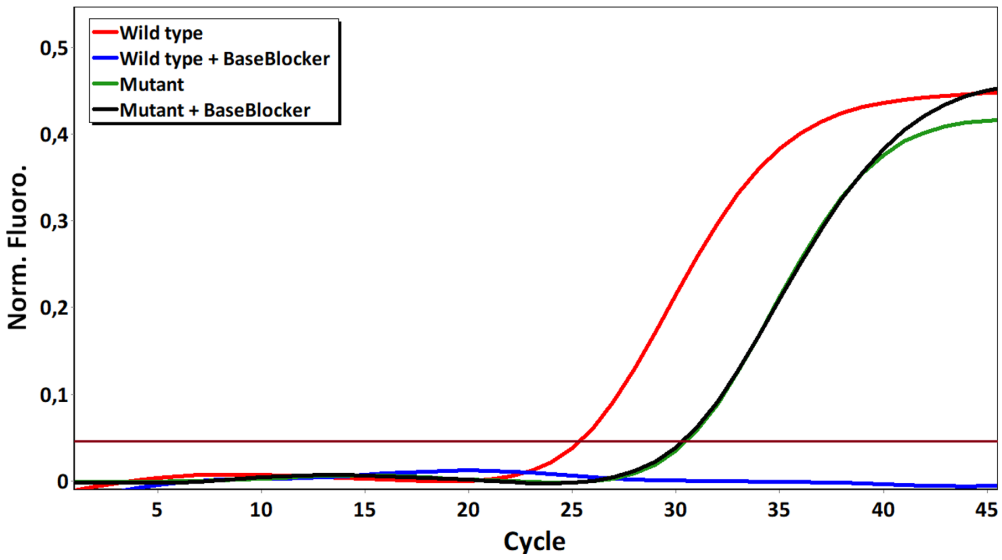


Illustration created with BioRender.com

BaseBlocker™s are short oligonucleotides that are highly modified with several IPNs. BaseBlocker™s have high target affinity and specificity, allowing sequence specific blocking of for example wild type templates*. In addition, BaseBlockers™ are modified to ensure that they do not work as potential primers.

BaseBlocker™s enable detection of down to a single DNA molecule with a single SNP in a background of wild-type DNA^, lowering the risk of false positive signals without influencing the rate of false negatives. The example shows how adding a BaseBlocker™ to an allele-specific qPCR Assay blocks amplification of the wild type allele (red and blue lines) without affecting amplification of the mutant allele (green and black lines).



BaseBlocker™ advantages

- INA® modified BaseBlocker™ for highly specific sequence discrimination
- Extremely sensitive mutant detection in wild-type background reducing the risk of false positives

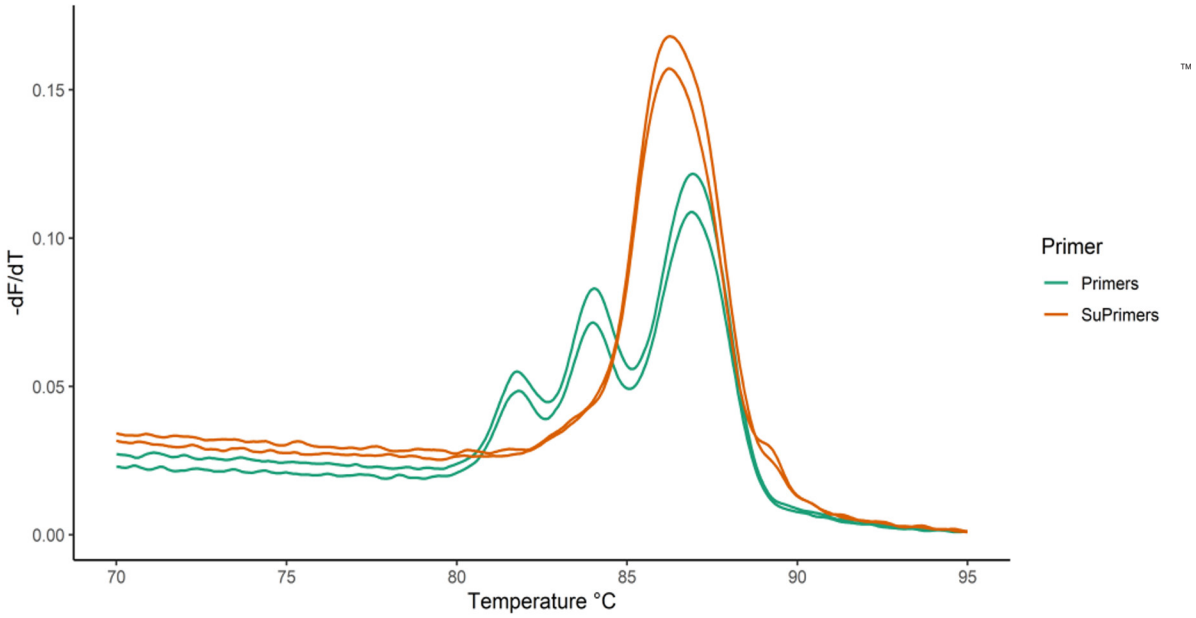
* 1. Riva A, et al. 2017. https://www.doi.org/10.1371/journal.pone.0178027
2. Sørensen AL, et al. 2023. https://www.doi.org/10.1371/journal.pone.0281558

^ Jensen SG, et al. 2021. https://www.doi.org/10.1371/journal.pone.0253687

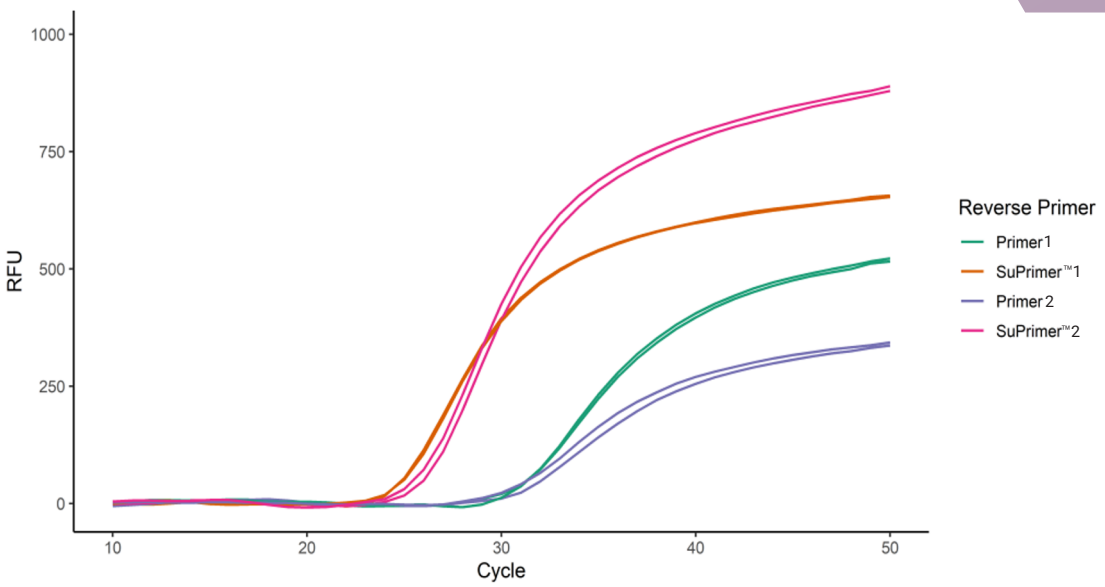
SuPrimer™

The INA® modified SuPrimer™ works as a normal primer in the PCR reaction, just better. It gives you the capability of avoiding primer-dimer formation and at the same time increasing affinity, specificity, sensitivity and signal strength.

Amplification using regular primers often results in unspecific products due to primer-dimer interactions and/or other off target events. The increased affinity and specificity of the SuPrimer™s greatly reduces the risk of these primer-dimer interactions and off-target events, resulting in amplification of only the target sequence.



The example above shows an end-point melting curve following amplification of the RNase P gene used as internal control in SARS-CoV-2 testing with the primers and SuPrimer™s both made on the basis of the WHO recommendations. The assay based on SuPrimer™s show a only single PCR product compared to several products when using standard DNA primers and also higher signal strength.



Including the SuPrimer™s in qPCR assay design can increase specificity, sensitivity and signal strength. The example above shows the difference between the amplification curves of two normal primer sets compared to two corresponding primer sets comprising a modified reverse SuPrimer™. Primer1 and SuPrimer™1 amplifies an identical sequence and the same applies to Primer2 and SuPrimer™2.

The use of SuPrimer™s results in higher sensitivity and higher fluorescent signals than the use of regular primers.

SuPrimer™ advantages

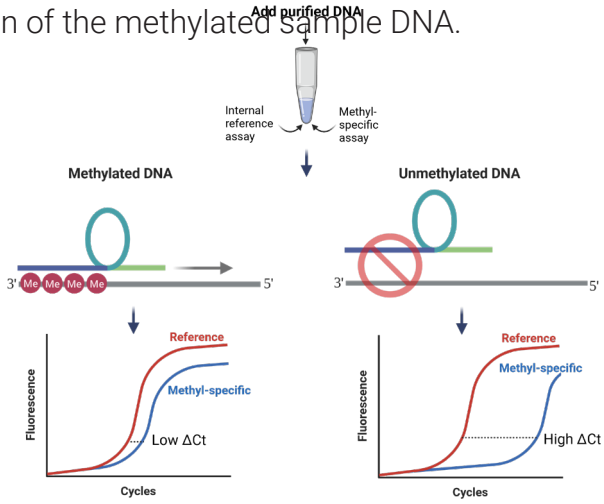
- Reduces primer-dimer formation
- Lower off-target binding and higher target specificity and affinity
- Higher signal strength

EpiDirect®

The EpiDirect® technology utilizes a type of INA® known as an EpiPrimer™ that allows for direct evaluation of DNA methylation by qPCR without prior chemical conversion (reference til Nature Genetics artiklen).

The EpiPrimer™ consists of 3 parts: a) an IPN-containing anchor sequence that binds to the methylated target region, b) a starter sequence that primes DNA replication, and c) a loop sequence that is part of the subsequent methyl-unspecific priming. Thus, the anchor and starter sequence prime the initial replication of the methylated DNA while the loop and starter sequence combined work as a primer in the remaining PCR cycles.

The high specificity of the IPN-containing anchor sequency towards the methylated target allows for highly selective amplification of the methylated sample DNA.



EpiDirect® advantages

- Direct evaluation of DNA methylation using customised INA®-based EpiPrimer™ design
- Avoids artefacts related to chemical conversion of DNA prior to real-time PCR

Custom PCR assays

As leading PCR experts, we have vast experience in developing and producing highly sensitive and specific assays for our customers. Our knowhow and unique INA™ technology allows us develop novel high performance PCR assays or significantly improve the performance of client assays previously based on standard nucleotide chemistries.

For our customized assays we have specific minimum quality standards for delivery and guarantied performance:

- Minimum 10 nmol delivered
- Validation will be performed on PentaBase Mastermix and PentaBase BasePurifier™ / BaseTyper™ instruments
- SNP genotyping assay (melt-based) will find 100 or less copies of synthetic SNP variant target in 5 ng synthetic wild-type background with a specificity determined by a temperature difference between variant and wild-type target of minimum 4 degrees
- Pathogen identification assay with a limit of detection (LOD) of 100 or less copies of synthetic template hidden in a background of 5 ng human genomic DNA
- Somatic mutation assays with a LOD of 1% or better measured using synthetic mutated templates hidden in a background of human genomic DNA
- Custom EpiDirect® Anchor Design specificity as determined by a difference in melting temperature between methylated and unmethylated target of at least 5°C
- Custom EpiDirect Assay Design limit of detection (LoD) of 5% methylation or better

How to order

Customised oligonucleotides and PCR assays can be ordered by sending an email to **order@pentabase.com**. When ordering custom oligonucleotides, please include the following information, and do not worry about including the IPNs in your design, our specialists will add IPNs once a query has been sent:

- Synthesis scale
- Sequence (5' to 3') + given sequence name
- 5' and 3' modifications
- Purification
- Technology

Synthesis scale
10 nmol delivered
50 nmol delivered
100+ nmol delivered (discounts apply)

Technology
EasyBeacon™ probe
HydrolEasy® probe
SuPrimer™
BaseBlocker™
EpiPrimer™
Conventional primer
Conventional probe

Purification
RPLC
HPLC
2x HPLC

Our custom oligonucleotides can be ordered in fixed amounts from 10 nmol delivered and supplied as either lyophilized or in aliquoted solutions as specified by the customer. We use several control steps to ensure oligonucleotide quality including measuring trityl data to calculate synthesis efficiency, evaluating HPLC spectra to ensure pure oligonucleotide output, and measuring optical densities for accurate concentration determination. In addition, we perform a target melting curve analysis of our probes to evaluate target binding properties.

Purification can be done through RPLC (cartridge purification) or RP-HPLC, and every order is sent with a synthesis report. A copy of the melt analysis can be included upon request.

Prices

Below is the price table for our custom oligonucleotides.

DNA Primer™ 14-39 mer		
Ref. No.	**Product name**	**List price**
430	DNA Primer; 10 nmol delivered; RPLC	*50,00 €*
431	DNA Primer; 10 nmol delivered; HPLC	*60,00 €*
432	DNA Primer; 50 nmol delivered; RPLC	*60,00 €*
433	DNA Primer; 50 nmol delivered; HPLC	*70,00 €*
DNA Primer™ 40-100 mer		
Ref. No.	**Product name**	**List price**
690	DNA Primer; 10 nmol delivered; HPLC	*120,00 €*
691	DNA Primer; 50 nmol delivered; HPLC	*140,00 €*
SuPrimer™ 14-35 mer		
Ref. No.	**Product name**	**List price**
434	SuPrimer™; 10 nmol delivered; HPLC	*90,00 €*
435	SuPrimer™; 50 nmol delivered; HPLC	*100,00 €*
BaseBlocker™ 14-35 mer		
Ref. No.	**Product name**	**List price**
436	Design of BaseBlocker™	*500,00 €*
437	BaseBlocker™; 10 nmol delivered; HPLC	*140,00 €*
438	BaseBlocker™; 50 nmol delivered; HPLC	*280,00 €*
EasyBeacon™ / HydrolEasy® Probe 14-35 mer; 10 nmol Delivered		
Ref. No.	**Product name**	**List price**
439/459	FAM/PentaGreen-BHQ1/Green Quencher/DPQ1	*160,00 €*
440/460	HEX/PentaYellow-BHQ1/Yellow Quencher/DPQ1	*180,00 €*
441/461	CalFluor Red/PentaOrange-BHQ2/ODPQ2	*230,00 €*
442/462	Atto 425/465-BHQ1/DPQ1	*230,00 €*
443/463	Atto 565-BHQ2/DPQ2	*230,00 €*
444/464	Quasar 670/Cy5/PentaRed-BHQ2/DPQ2	*300,00 €*
445/465	Atto 390/495-BHQ1/DPQ1	*300,00 €*
446/466	Quasar 705/Cy5.5/PentaPurple-BHQ2/DPQ2	*350,00 €*
447/467	Atto 488/490/532/550-BHQ1/DPQ1	*460,00 €*
448/468	Atto 594/647N/700/725-BHQ2/BBQ-650/DPQ2	*460,00 €*

EasyBeacon™ / HydrolEasy® Probe 14-35 mer; 50 nmol Delivered		
Ref. No.	Product name	List price
449/469	FAM/PentaGreen-BHQ1/Green Quencher/DPQ1	*240,00 €*
450/470	HEX/PentaYellow-BHQ1/Yellow Quencher/DPQ1	*300,00 €*
451/471	CalFluor Red/PentaOrange-BHQ2/ODPQ2	*480,00 €*
452/472	Atto 425/465-BHQ1/DPQ1	*490,00 €*
453/473	Atto 565-BHQ2/DPQ2	*650,00 €*
454/474	Quasar 670/Cy5/PentaRed-BHQ2/DPQ2	*650,00 €*
455/475	Atto 390/495-BHQ1/DPQ1	*660,00 €*
456/476	Quasar 705/Cy5.5/PentaPurple-BHQ2/DPQ2	*700,00 €*
457/477	Atto 488/490/532/550-BHQ1/DPQ1	*920,00 €*
458/478	Atto 594/647N/700/725-BHQ2/BBQ-650/DPQ2	*920,00 €*
DNA Probe 14-35 mer; 10 nmol Delivered		
Ref. No.	Product name	List price
479	FAM/PentaGreen-BHQ1/Green Quencher/DPQ1	*130,00 €*
480	HEX/PentaYellow-BHQ1/Yellow Quencher/DPQ1	*150,00 €*
481	CalFluor Red/PentaOrange-BHQ2/ODPQ2	*200,00 €*
482	Atto 425/465-BHQ1/DPQ1	*200,00 €*
483	Atto 565-BHQ2/DPQ2	*200,00 €*
484	Quasar 670/Cy5/PentaRed-BHQ2/DPQ2	*270,00 €*
485	Atto 390/495-BHQ1/DPQ1	*270,00 €*
486	Quasar 705/Cy5.5/PentaPurple-BHQ2/DPQ2	*320,00 €*
487	Atto 488/490/532/550-BHQ1/DPQ1	*430,00 €*
488	Atto 594/647N/700/725-BHQ2/BBQ-650/DPQ2	*430,00 €*
DNA Probe 14-35 mer; 50 nmol Delivered		
Ref. No.	Product name	List price
489	FAM/PentaGreen-BHQ1/Green Quencher/DPQ1	*180,00 €*
490	HEX/PentaYellow-BHQ1/Yellow Quencher/DPQ1	*240,00 €*
491	CalFluor Red/PentaOrange-BHQ2/ODPQ2	*420,00 €*
492	Atto 425/465-BHQ1/DPQ1	*430,00 €*
493	Atto 565-BHQ2/DPQ2	*430,00 €*
494	Quasar 670/Cy5/PentaRed-BHQ2/DPQ2	*590,00 €*
495	Atto 390/495-BHQ1/DPQ1	*600,00 €*
496	Quasar 705/Cy5.5/PentaPurple-BHQ2/DPQ2	*640,00 €*
497	Atto 488/490/532/550-BHQ1/DPQ1	*860,00 €*
498	Atto 594/647N/700/725-BHQ2/BBQ-650/DPQ2	*860,00 €*

Discounts - Oligonucleotides*	
nmol delivered	Discount rate
100 nmol	10%
250 nmol	20%
500 nmol	25%
1000 nmol	30%
2000 nmol	35%

*Discounts are applied to 50 nmol delivered based on order volume

Below is the price table for our New Assay Services.

NEW SNP ASSAY (one base change)		
Ref. No.	**Product name**	**List price**
620	New SNP genotyping simplex assay design	*2.460,00 €*
621	New SNP genotyping duplex assay design	*5.730,00 €*
622	New SNP genotyping triplex assay design	*8.990,00 €*
623	New SNP genotyping quadruplex assay design	*11.860,00 €*
625	New SNP genotyping simplex assay verification	*3.620,00 €*
626	New SNP genotyping duplex assay verification	*8.660,00 €*
627	New SNP genotyping triplex assay verification	*13.690,00 €*
628	New SNP genotyping quadruplex assay verification	*18.120,00 €*
	New SNP genotyping assay custom services (specific performance requirements, performance evaluation, etc.)	*Inquire*
NEW PATHOGEN IDENTIFICATION ASSAY		
Ref. No.	**Product name**	**List price**
635	New simplex pathogen identification assay design	*2.460,00 €*
636	New duplex pathogen identification assay design	*5.730,00 €*
637	New triplex pathogen identification assay design	*8.990,00 €*
638	New quadruplex pathogen identification assay design	*11.860,00 €*
640	New simplex pathogen identification assay verification	*3.620,00 €*
641	New duplex pathogen identification assay verification	*8.660,00 €*
642	New triplex pathogen identification assay verification	*13.690,00 €*
643	New quadruplex pathogen identification assay verification	*18.120,00 €*
	New pathogen identification assay custom services (specific performance requirements, performance evaluation, etc.)	*Inquire*
NEW SOMATIC MUTATION ASSAY		
Ref. No.	**Product name**	**List price**
575	New simplex somatic mutation assay design	*3.020,00 €*
576	New duplex somatic mutation assay design	*6.850,00 €*
577	New triplex somatic mutation assay design	*10.880,00 €*
578	New quadruplex somatic mutation assay design	*14.510,00 €*
580	New simplex somatic mutation assay verification	*3.620,00 €*
581	New duplex somatic mutation assay verification	*8.660,00 €*
582	New triplex somatic mutation assay verification	*13.690,00 €*
583	New quadruplex somatic mutation assay verification	*18.120,00 €*
	New somatic mutation assay custom services (specific performance requirements*, performance evaluation, etc.)	*Inquire*

CUSTOM EpiDirect® DESIGN		
Ref. No.	**Product name**	**List price**
660	Custom EpiDirect® Anchor Design and Validation	*3,630.00 €*
661	Custom EpiDirect® Assay Design and Validation	*29,330.00 €*

Below is the price table for our Custom Individual Services.

CUSTOM INDIVIDUAL SERVICES		
Ref. No.	**Product name**	**List price**
	Custom assay analytical performance evaluation IVDR Stage 4 - Accuracy[1], Analytical Sensitivity[2] and Analytical Specificity[3]	*Inquire*
	Custom EpiDirect® Assay Optimisation[4]	*Inquire*
	[1]*Accuracy* including *Trueness* and *Precision* *Trueness* will be determined by calculating limits of agreement (LOA) using relevant available certified reference materials and at least 20 measurement points. *Precision* will include repeatability (n≥ 20) and within laboratory reproducibility for up to 3 factors (lots, time, instruments, etc.) [2]*Analytical sensitivity* will include limit of blank (LoB), limit of detection (Lod) and limit of quantitation (LoQ) when relevant, of at least 20 measurement points. [3]*Analytical specificity* will include relevant in silico analyses and wet lab testing of up to 5 interfering targets or substances. [4]Oligonucleotide titration, PCR program optimization, etc. All analyses require availability of clinically relevant sample material (FFPE, plasma, etc.) and related costs are not included. Data will be summarised in an analytical performance evaluation report	

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PentaBase

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For further information, visit
www.pentabase.com