IBIAN LAB

PRODUCT CATALOG

IBIAN LAB

Convenient and quality biotech tools

IBIANLAB PRODUCTS

Contenido

Taq DNA Polymerase

Ref.	Product	Type	DNA Polymerases	HotStart	Proof reading Activity	5'3' Exon. Activity
i01-105	IBIAN-Taq DNA Polymerase 5 U/ µl (500 U)	Standard	Standard PCR Bacterial DNA detection	No	No	Yes
i01-105X5	IBIAN-Taq DNA Polymerase 5 U/ µl 2500 U (5 tubes)					
Ref.	**Product**	**Type**	**DNA Polymerases**	**HotStart**	**Proof reading Activity**	**5'3' Exon. Activity**
i01-HT05	IBIAN®-HighHotTaq DNA Polymerase 500 U	HotStart	Standard PCR Multiplex PCR qPCR	Yes	No	Yes
i01-HT05x5	IBIAN®-HighHotTaq DNA Polymerase 2500 U (5 tubes)					
i01-H02	IBIAN-HotTaq DNA Polymerase 200 U	HotStart	Standard PCR Multiplex PCR qPCR	Yes	No	Yes
i01-H02x5	IBIAN-HotTaq DNA Polymerase 1000 U (5 tubes)					

Master Mix

Ref. No	Tested Master Mix	Standard PCR	Multiplex PCR	qPCR	Hot Start	Dye
i01-202	IBIAN-Taq Master Mix 200 rcs (2 x 1.25 ml tube)	Yes	No	No	No	No
I01-202X5	IBIAN-Taq Master Mix 1000 rcs (10 x 1.25 ml tube)					
i01-M02	IBIAN-HotTaqMaster Mix 200 rcs (2 tubes x 1.25 ml)	Yes	Yes	Yes	Yes	No
i01-M02x5	IBIAN-HotTaqMaster Mix 1000 rcs (10 tubes x 1.25 ml)					

Dyes

Ref.	Type of Dye	Pack Size
I05-G100	ibGreen 100x for Real-Time PCR 100 µl	1 tube of 100 µl
I05-G100X5	ibGreen 100x for Real-Time PCR 100 µl	5 tube of 100 µl
i05-G10000A	IBIAN®-ibGreenGel Staining Solution 50 µL	1 tube of 50 µl
i05-G10000B	IBIAN®-ibGreenGel Staining Solution 500 µL	1 tube of 500 µl
i05-G10000C	IBIAN®-ibGreenGel Staining Solution 1000 µL	1 tube of 1000 µl
I05-LB05	LOADING BUFFER II (6X)	5 x 1 ml (100 mg)

dNTPs

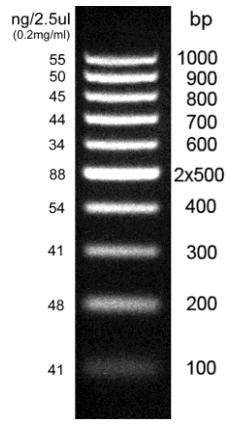
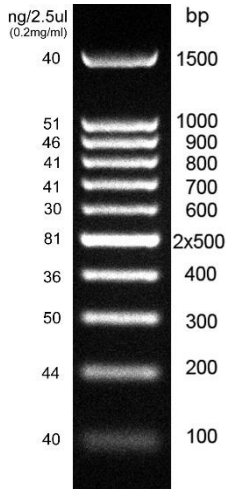
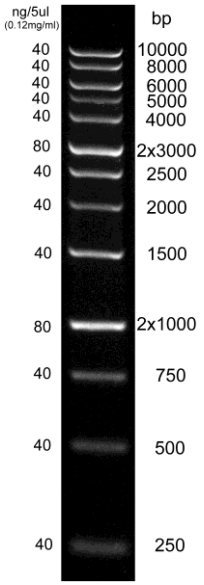
Ref.	Tested Master Mix	Pack Size
i02-M02	IBIAN 4you4 dNTP Mix, 10 mM each	(1 x 1000 µl)
i02-M02X5	IBIAN 4you4 dNTP Mix, 10 mM each	(5 x 1000 µl)
i02-S11	IBIAN Set of 4 dNTPs, 4 x 100 mM	(4 x 200 µl)
i02-S22	IBIAN-Set of 4 dNTPs, 100 mM 4 x 1000 µl	(4 x 1000 µl)

Proteinase K

Ref.	Proteinase K Solution	Pack Size
i06-PK01	Proteinase K Solution	1 ml (20 mg)
i06-PK01x5	Proteinase K Solution 20 mg/ml	5 x 1 ml (100 mg)

DNA Size Ladder

Ref. No	Ladder	DNA Size	Pack Size	With Stain
i03-L05	IBIAN 100 bp DNA Ladder no stain	100 bp	50 µg	NO
i03-B05	IBIAN 100 bp DNA **Ladder ready to use (Blue)**	100 bp	50 µg	Yes
i03-B05P	IBIAN 100 bp **PLUS** DNA **Ladder ready to use (Blue)**	100 bp Plus	50 µg	Yes
i03-L10	IBIAN 1 kb DNA Ladder no stain	1 kb	50 µg	NO
i03-B10	IBIAN 1 kb DNA **Ladder ready to use (Blue)**	1 kb	50 µg	Yes

100 bp Ladder	100 bp plus Ladder	1 kb Ladder
		
100 bp –1000 bp 10 fragments	100 bp – 1500 bp 11 fragments	250 –10000 bp 13 fragments

IBIAN®-Taq DNA Polymerase

Concentration: 5 units/µl

Description

IBIAN®-Taq DNA Polymerase is a thermostable enzyme of approximately 94 kDa, isolated from eubacterium Thermus aquaticus strain YT-1. This unmodified enzyme replicates DNA at 72 °C. The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium ions and shows 5' → 3' exonuclease activity. The enzyme is highly purified and free of nonspecific endo- or exonucleases. IBIAN-Taq DNA Polymerase leaves single 3'-A overhang nucleotides on their reaction products.

Shipping and Storage Conditions

Product is shipped on blue-ice.
Store at -20 °C Avoid repeated freeze/thaw cycles. Reagent for in vitro laboratory use only

Content and Order information

Catalog No.:	i01-105	i01-105X5
IBIAN®-Taq DNA Polymerase	500 units	2500 units
Incomplete NH_4 [a] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
Complete NH_4 [b] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
Complete KCl [c] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
$MgCl_2$ 100 mM	1ml	5x 1ml
Datasheet	1	1

[a] Incomplete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, free of $MgCl_2$.

[b] Complete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 25 mM $MgCl_2$.

[c] *Complete KCl Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 15 mM $MgCl_2$.

Performance and purity tests

IBIAN®-Taq DNA Polymerase effectively directs PCR with templates up to 5 kb in length. The enzyme was tested for the absence of exo-/endonuclease activities. No traces of bacterial DNA were detected in PCR reaction with “no template” test with the primers complementary to the conservative region of 16S ribosomal gene.
The following tests are performed with each lot of IBIAN-Taq DNA Polymerase:

- PCR amplification tests with different templates
- No detectable exo-/endonuclease activities
- “no primers” test with Lambda DNA cycling without primers
- “no template” test with primers complementary to a region of 16S bacterial ribosomal genes.

Applications

IBIAN®-Taq DNA Polymerase is suitable for all regular applications – PCR, primer extension reactions etc. IBIAN®-Taq is free of bacterial DNA and therefore suitable for working with bacterial DNA.

Sensitivity

High sensitivity of PCR reactions with IBIAN®-Taq DNA polymerase in the optimal conditions – in some reactions at least 10 DNA molecules are necessary for detection. Enzyme has a very good performance in single-copy gene PCR from genomic mammalian DNA. In contrast to the IBIAN enzyme, Taq DNA polymerases from the variety of suppliers contain contaminating DNA. With DNA-contaminated Taq DNA polymerase you can observe false-positive PCR results in some cases.

Unit definition: One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72 °C.

Recommended $MgCl_2$ concentration 1.5 mM – 6 mM

Storage buffer:
Enzyme is supplied in 10mM K3PO4 pH 7.4, 0.1mM EDTA, 50% glycerol, 0.1% Triton X-100, 0.1% Tween-20.

Pipetting scheme

This protocol serves as a guideline for PCR. Optimal reaction conditions may vary and must be determined individually.

Components	Volume / 50 µl PCR-Reaction	Final concentration
10 x PCR-Buffer	5 µl	1 x
dNTP-Mix (10 mM each)	1 µl (see ref. I02-M02)	800 µM (200 µM each)
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
IBIAN®-Taq DNA Polymerase	0.25 - 1.0 µl	1.25 - 5.0 units
Template DNA	variable	10 to 500 ng/reaction
Sterile PCR water	Adjust to 50 µl final volume	

Separate MgCl2 solution can be used for optimization. If incomplete buffer is used titrate MgCl2 for optimal PCR results with following recommendation (see table):

Final MgCl2 conc. [mM]									
1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
0.75	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3
Volume [µL] of 100 mM MgCl2/ 50 µL									

Thermocycler sample protocol

Step	Time	Temperature
Initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C
[a]Final Elongation	*5 minutes*	*72 °C*

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

[a] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining singlestranded DNA is fully extended.

IBIAN®-HighHotTaq DNA Polymerase

Concentration: 5 units/µl

Description

IBIAN®-HighHotTaq DNA Polymerase is a thermostable enzyme of approximately 94 kDa, isolated from eubacterium Thermus aquaticus strain YT-1 bound to anti-Taq DNA polymerase monoclonal antibodies. Polymerase activity is blocked during set-up of the PCR at ambient temperature (20 – 22 °C). The inhibition is completely reversed when the temperature is increased above 70 °C The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5' → 3' direction in the presence of magnesium ions and shows 5' → 3' exonuclease activity. The enzyme is highly purified and free of nonspecific endo- or exonucleases.

Shipping and Storage Conditions

Product is shipped on blue-ice.
Store at -20 °C Avoid repeated freeze/thaw cycles. Reagent for in vitro laboratory use only

Content and Order information

Catalog No.:	**i01-HT05**	**i01-HT05X5**
IBIAN®-HighHotTaq DNA Polymerase	**500 units**	**2500 units**
Incomplete NH_4 [a] Reaction Buffer (10x)	**1.8 ml**	**5x 1.8 ml**
Complete NH_4 [b] Reaction Buffer (10x)	**1.8 ml**	**5x 1.8 ml**
Complete KCl [c] Reaction Buffer (10x)	**1.8 ml**	**5x 1.8 ml**
$MgCl_2$ 100 mM	**1ml**	**5x 1ml**
Datasheet	**1**	**1**

[a] Incomplete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, free of $MgCl_2$.

[b] Complete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 25 mM $MgCl_2$.

[c] *Complete KCl Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 15 mM $MgCl_2$.

Performance and purity tests

IBIAN®-HighHotTaq DNA Polymerase effectively directs PCR with templates up to 5 kb in length. The enzyme was tested for the absence of exo-/endonuclease activities. No traces of bacterial DNA were detected in PCR reaction with “no template” test with the primers complementary to the conservative region of 16S ribosomal gene.
The following tests are performed with each lot of IBIAN-HighHotTaq DNA Polymerase:

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- “no template” test with primers complementary to a region auf 16S bacterial ribosomal genes
- Hotstart efficiency test showing effective blockage by AntiTaq

Applications

IBIAN®-HighHotTaq DNA Polymerase is suitable for all regular applications – PCR, Multiplex PCR, primer extension reactions etc. IBIAN®-Taq is free of bacterial DNA and therefore suitable for working with bacterial DNA.

Sensitivity

High sensitivity of PCR reactions with IBIAN®-HighHotTaq DNA Polymerase in the optimal conditions – in some reactions at least 10 DNA molecules are necessary for detection. Enzyme has a very good performance in single-copy gene PCR from genomic mammalian DNA. In contrast to the IBIAN enzyme, HighHotTaq DNA Polymerases from the variety of suppliers contain contaminating DNA. With DNA-contaminated HighHotTaq DNA Polymerase you can observe false-positive PCR results in some cases.

Unit definition: One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72 °C.

Recommended $MgCl_2$ concentration 1.5 mM – 6 mM

Pipetting scheme

This protocol serves as a guideline for PCR. Optimal reaction conditions may vary and must be determined individually.

Components	Volume / 50 µl PCR-Reaction	Final concentration
10 x PCR-Buffer	5 µl	1 x
dNTP-Mix (10 mM each)	1 µl (see ref. I02-M02)	800 µM (200 µM each)
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
IBIAN®-HighHotTaq DNA Polymerase	0.25 - 1.0 µl	1.25 - 5.0 units
Template DNA	variable	10 to 500 ng/reaction
Sterile PCR water	Adjust to 50 µl final volume	

Separate MgCl2 solution can be used for optimization. If incomplete buffer is used **titrate $MgCl_2$** for optimal PCR results with following recommendation (see table):

Final $MgCl_2$ conc. [mM]									
1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
0.75	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3
Volume [µL] of 100 mM $MgCl_2$/ 50 µL									

Thermocycler sample protocol

Step	Time	Temperature
Initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C
[a]*Final Elongation*	*5 minutes*	*72 °C*

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

[a] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining singlestranded DNA is fully extended.

IBIAN®-HotTaq DNA Polymerase

Concentration: 5 units/µl

Description

IBIAN®-HotTaq DNA Polymerase is the optimized mixture of Taq DNA polymerase and anti-Taq DNA polymerase monoclonal antibodies. Polymerase activity is blocked during set-up of the PCR reactions at ambient temperature (20 – 22 °C) by antibodies. The inhibition of Taq DNA polymerase is completely reversed when the temperature increased above 70 °C. The PCR products obtained with IBIAN®-HotTaq DNA Polymerase are free of unspecific products and primer-dimers.

Shipping and Storage Conditions

Product is shipped on blue-ice.
Store at -20 °C Avoid repeated freeze/thaw cycles. Reagent for in vitro laboratory use only

Content and Order information

Catalog no.	i01-H02	i01-H02X5
HotTaq DNA Polymerase	200 units	1000 units
Incomplete NH_4 [a] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
Complete NH_4 [b] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
Complete KCl [c] Reaction Buffer (10x)	1.8 ml	5x 1.8 ml
$MgCl_2$ 100 mM	1ml	5x 1ml
Datasheet	1	1

[a] Incomplete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, free of $MgCl_2$.

[b] Complete NH_4 Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 25 mM $MgCl_2$.

[c] *Complete KCl Reaction Buffer (10x): pH 8.8, 0.1% Tween 20, 15 mM $MgCl_2$.

Performance and purity tests

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- PCR amplification tests without templates as Negative Control
- Hotstart efficiency test showing effective blockage by AntiTaq
- Exonuclease efficiency test showing efficient 5´ - 3´Exonuclease activity.

Applications

IBIAN®-HotTaq DNA Polymerase is suitable for all regular applications but especially for PCR with complex genomic or cDNA templates, low copy number targets, large number of thermal cycles, Multiplex and Real Time PCR. IBIAN®HotTaq DNA Polymerase effectively directs PCR with the template up to 5 kb in length.

Sensitivity

High sensitivity of PCR reactions detection of ≥ 6 DNA molecules

Unit definition: One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72 °C.

Recommended $MgCl_2$ concentration 1.5 mM – 6 mM

Additionally provided: 1 tube MgCl2 (100 mM)

Pipetting scheme

This protocol serves as a guideline for PCR. Optimal reaction conditions may vary and must be determined individually.

Components	Volume / 50 µl PCR-Reaction	Final concentration
10 x PCR-Buffer	5 µl	1 x
dNTP-Mix (40 mM)	1 µl	200 µM each
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
IBIAN®-HotTaq DNA Polymerase	0.25 - 1.0 µl	1.25 - 5.0 units
Template DNA	variable	10 to 500 ng/reaction
Sterile PCR water	Adjust to 50 µl final volume	

Separate MgCl2 solution can be used for optimization. If incomplete buffer is used titrate MgCl2 for optimal PCR results with following recommendation (see table):

Final MgCl2 conc. [mM]									
1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
0.75	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3
Volume [µL] of 100 mM MgCl2/ 50 µL									

Thermocycler sample protocol

Step	Time	Temperature
Initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C
[a]*Final Elongation*	*5 minutes*	*72 ºC*

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

[a] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining singlestranded DNA is fully extended.

Notes:
Program the cycler according to the manufacturer's instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 1 min/ 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

IBIAN®- Taq Master Mix

Concentration: 2x

Description

IBIAN®-Taq Master Mix is an optimized ready-to-use PCR mixture of Taq DNA Polymerase, PCR buffer, MgCl2 and dNTPs. IBIAN®-Taq Master Mix contains all components for PCR, except DNA template and primers

Shipping and Storage Conditions

Product is shipped on blue-ice.
Store at -20 °C Avoid repeated freeze/thaw cycles. Reagent for in vitro laboratory use only

Content and Order information

Catalog no.	i01-202	i01-202x5
IBIAN-Taq Master Mix (2x) * (White)	200 reactions 2x1.25 ml	1000 reactions 10x1.25 ml
PCR Water	2x1.8 ml	10x1.8 ml
$MgCl_2$ 100 mM	1ml	5x 1ml
Datasheet	1	1

*Contains Taq DNA Polymerase (recombinant), PCR Buffer with 5.5 mM MgCl2 and 400 µM each dNTP.

Performance and purity tests

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates

Applications

IBIAN®-Taq Master Mix is suitable for a wide range of PCR methods like qPCR, Real-Time PCR and classic PCR. It can be used for regular PCR with a fragment-size up to 5 kb.

Sensitivity

High sensitivity of PCR reactions

Unit definition:

One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 72 °C.

Recommended MgCl2 concentration:

1.5 mM – 6 mM **Additionally provided:** 1 tube MgCl2 (100 mM)

Pipetting scheme

25µl reaction volume		
Component	**Volume**	**Final concentration**
IBIAN®-Taq Master Mix	12.5µl	1X
Forward Primer	variable	0.1-1µM
Template DNA	variable	100pg-1µg
Template DNA	variable	100pg-1µg
Sterile Deionized Water	up to 25µl	-

It's strongly recommended to settle-up all reactions on ice to avoid formation of unspecific products or primer-dimers formation.
If MgCl2 is not added to the reaction mixture, final concentration of MgCl2 in the reaction mixture will be 2mM.

Thermocycler sample protocol

Step	**Time**	**Temperature**
Initial denaturation	2 minutes	94 °C
Number of cycles: 25 - 35		
denaturation	10- 30 seconds	94 °C
annealing	20 - 30 seconds	55 - 68 °C *
extension	1 minute	72 °C
[a]*Final Elongation*	*5 minutes*	*72 °C*

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

[a] Final elongation: This single step is occasionally performed at a temperature of 72 °C for 5 minutes after the last PCR cycle to ensure that any remaining singlestranded DNA is fully extended.
Notes:
Program the cycler according to the manufacturer's instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 1 min/ 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

IBIAN®-HotTaq Master Mix

Concentration: 2x

Description: IBIAN®-HotTaq Master Mix is an optimized ready-to-use PCR mixture of Taq DNA Polymerase, antibodies to Taq DNA polymerase, PCR buffer, MgCl2 and dNTPs. 2x PCR Master Mix contains all components for PCR, except DNA template and primers. The mixture was shown to be effective for Real Time PCR

Content

IBIAN®-HotTaqMaster Mix **Catalog no.**	**200 reactions** **i01-M02**	**1000 reactions** **i01-M02x5**
HotTaqMaster Mix (2x) *	**2x 1.25 ml**	**10x 1.25 ml**
MgCl2 100 mM	**1 ml**	**5x 1ml**
PCR Water	**2x 1.8 ml**	**10x 1.8 ml**
Datasheet	**1**	**1**

* Contains Antibody blocked Hotstart Taq DNA Polymerase (recombinant). NH_4 PCR Buffer with 3 mM $MgCl_2$ and 400 µM each dNTP.

Features
• High sensitivity & specificity
• HotTaq DNA Polymerase of IBIAN® included (Taq Polymerase with antibodies versus Taq polymerase)

Applications
• Real-Time qPCR assays
• PCR with End point analysis

Storage condition Store at -20°C. Avoid repeated freeze/thaw cycles. Product is shipped on blue-ice.

Performance and purity tests
Tested for the absence of endodeoxyribonucleases and exodeoxyribonucleases.
The HotTaqMaster Mix is tested in the amplification of a single-copy gene of mouse genomic DNA.

Endodeoxyribonuclease Assay: No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 25µl of IBIAN®-HotTaqMaster Mix with 1µg of pUC19 DNA in 50µl for 4 hours neither at 37°C nor at 70°C

IBIAN®-HotTaqMaster Mix is twofold concentrated 2 × 1.25ml of Master Mix is enough for 200 rcs in 25 µl final reaction volume.

Protocol for PCR with IBIAN®-HotTaqMaster Mix
Due to the inhibition of polymerase activity at room temperature by Anti Taq DNA polymerase antibodies all reactions may be settled-up at room temperature, it will not result in increase of unspecific product or primer-dimers formation.

Add in a thin walled PCR tube:

25µl reaction volume		
Components	**Volume**	**Final concentration**
HotTaq Master Mix	12,5 µl	1 x
Forward Primer	variable	0.1-1µM
Reverse Primer	variable	0.1-1µM
Template DNA	variable	100pg-1µg
Sterile Deionized Water	up to 25µl	up to 25µl

- Gently vortex the sample and briefly centrifuge to collect all drops to the bottom of the tube.
- Overlay the sample with mineral oil or add an appropriate amount of wax if the thermal cycler is not equipped with a heated lid.
- Place the samples in a thermocycler and start a PCR program

Real-time PCR amplification is done on ABI®PRISM 7700, iQ5™ Real Time PCR System (BioRad) or other appropriate machines for Real-time PCR suitable for use of intercalating dye chemistry. All samples are run in triplicate with the appropriate single PCR controls (no template, no primers). Always prepare 2 Master Mixes for gene of interest and control gene to be sure in experiment-to-experiment consistency.

Thermocycler protocol

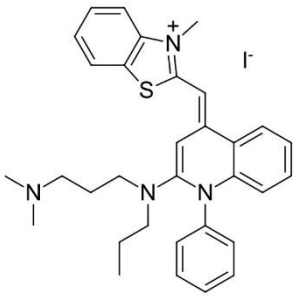
step	time	temperature
initial denaturation	2 minutes	94 °C
30 cycles:		
denaturation	10 seconds	94 °C
annealing	20 seconds	55 - 68 °C *
extension	1 minute	72 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers

Notes:
Program the cycler according to the manufacturers instructions. Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time is 1 min per 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

IBIAN®-ibGreen 100x for Real-Time PCR

Description



ibGreen, an analog of SYBR® Green I, is a very sensitive dsDNA detection dye. High sensitivity, and high selectivity for dsDNA allow to use ibGreen as a universal dsDNA detection reagent for qPCR. No need to use labeled probes to detect amplification with ibGreen – unlabeled primers are sufficient.
This formulation is specially designed to be used in real-time PCR experiments.
Specific features are:

- Concentration of the dye is optimized for qPCR and carefully adjusted for reproducible results from lot to lot.
- PCR tested preparation
- Low fluorescence background – high fluorescence intensity gain

Shipping and Storage Conditions

Product is shipped at RT.
Storage at -20°C in the dark. Avoid repeated freeze/thaw cycles.

Content and Order information

Catalog No.:	i05-G100	i05-G100X5
IBIAN®-ibGreen 100X	100 µl	5 x 100 µl
Datasheet	1	1

Protocol for qPCR with ibGreen 100x

If the ibGreen reagent was stored below 0 C, defrost it and keep it at room temperature (for rapid thawing, the reagent can be heated up to 50 C).

Calculate the volumes of reagents required for the reaction according to the manufacturer's instructions to PCR reagents. Please note that the ibGreen (100x in stock) must be diluted in a PCR end reaction volume up to 1x concentration.

Prepare a PCR mix containing no DNA according to the manufacturer's instructions to PCR reagents, add necessary amount of ibGreen (final concentration must be 1X). Keep the master mix and PCR tubes on the ice if you use Taq-polymerase without hot start.

Transfer the complete PCR master mix to tubes or plates and add DNA.

Proceed with amplification according to your instrument manufacturer.

Applications

IBIAN®-ibGreen 100x, is a very sensitive dye for the detection of double stranded DNA (dsDNA). It is used for non-specific detection of amplification in Real-Time qPCR experiments.

Please note that the ibGreen (100x in stock) must be diluted in a PCR end reaction volume up to 1x concentration.

Always include positive and negative controls in your qPCR experiments.

In order to be able to distinguish a specific product from primer dimers, use the melting curve step in your PCR program.

Pipetting scheme

This protocol serves as a guideline for qPCR using IBIAN-HotTaqMaster Mix as an example. Optimal reaction conditions may vary and must be determined individually.

Add in a thin walled PCR tube following components:

Components	Volume / 25 µl PCR-Reaction	Final concentration
IBIAN-HotTaqMaster Mix 2x (ref. I01-M02)	12.5 µl	1 x
Forward Primer	variable	0.1 - 0.5 µM
Revers Primer	variable	0.1 - 0.5 µM
Template DNA	variable	10 to 500 ng/reaction
ibGreen 100x	0.25 ul	1X
Sterile PCR water	Adjust to 25 µl final volume	

In IBIAN-HotTaqMaster Mix 2x If no additional MgCl2 is added to the reaction mixture, final concentration of MgCl2 in the reaction mixture will be 3 mM.

Thermocycler sample protocol

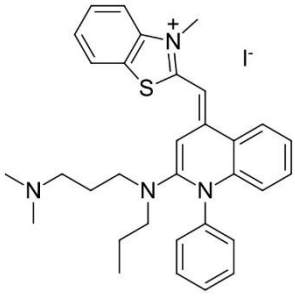
Step	Time	Temperature
Initial denaturation Enzyme activation	5 minutes	95 °C
30 - 45 cycles:		
denaturation	15 seconds	95 °C
annealing	60 seconds	60 °C *

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

In order to be able to distinguish a specific product from primer dimers, use the melting curve step in your PCR program.

IBIAN®-ibGreenGel Staining Solution

Description



ibGreenGel is structurally identical to SYBR® Green I Nucleic Acid Gel Stain, is one of the most sensitive stains available for detecting double-stranded DNA (dsDNA) in agarose and polyacrylamide gels. High sensitivity, and high selectivity for dsDNA allow to use ibGreenGel as a universal dsDNA detection reagent for DNA Staining in Gel.

Ref.	Quantity
i05-G10000A	IBIAN®-ibGreenGel Staining Solution 50 µL
i05-G10000B	IBIAN®-ibGreenGel Staining Solution 500 µL
i05-G10000C	IBIAN®-ibGreenGel Staining Solution 1000 µL

Feature	I05-G10000
Fluorescence	Green (524 nm)
Excitation maximum	454 nm
Excitation light source	Blue light or UV
Sensitivity	0.08 ng / band (dsDNA) 1–2 ng / band (oligonucleotides)
Health hazard	Low

SYBR is a registered trademark of Thermo Fisher Scientific.

Storage: 24 months after receival at -20°C in the dark.

Transportation: at room temperature for up to 3 weeks. Avoid prolonged exposure to light.

There are three variants of the staining protocol: Gel soaking, Gel pre-staining, and sample pre-staining.

Gel soaking

Classical method for agarose and polyacrylamide gels.

- Run sample(s) in an agarose or polyacrylamide gel.
- In a beaker, add 10 µL of the 10,000× ibGreen solution in DMSO to 100 mL of 1× TE, TBE, or TAE buffer (for mini gels), or 50 µL of the 10,000× ibGreen solution in DMSO to 500 mL of 1× TE, TBE, or TAE buffer (for mid-sized gels). Mix thoroughly with a spatula, rod, or magnetic stirrer.
- Pour the diluted ibGreen solution into an appropriate tray or pan and submerge the gel.
- Soak the gel for 5–10 min.

- View or document the gel using available light source and an green/yellow filter. Transilluminators with blue light, or with UV low pressure mercury lamp (254 nm) can be used to visualize gels stained with ibGreen. High pressure mercury lamp (365 nm) can be used too, but this light source gives somewhat less efficient excitation.

Gel pre-staining

This method is suitable for agarose gels only, but not for PAAG. Note - this staining method can sometimes cause bands to warp or form smears. Use gel soaking in this case.

- Boil the agarose in buffer to dissolution using a microwave or heating appliance.
- While still fluid, add 1 µL of the 10,000× ibGreen solution in DMSO per each 10 mL of gel solution. Mix thoroughly.
- Pour the gel and let it solidify.
- For best results, add 1 µL of of the 10,000× ibGreen solution in DMSO per each 10 mL of buffer near the anode ("+", red wire).
- Run the samples. Real-time monitoring of migrating bands under a 254 nm low-pressure mercury lamp is possible.
- View or document the gel using available light source and an green/yellow filter. Transilluminators with blue light, or with UV low pressure mercury lamp (254 nm) can be used to visualize gels stained with ibGreen. High pressure mercury lamp (365 nm) can be used too, but this light source gives somewhat less efficient excitation.

DNA Staining in Gel

Least sensitive, most economical method.

- Mix 25 µL of DMSO and 1 µL of the 10.000× ibGreen solution in DMSO.
- Add 1 µL of the solution to each sample to be separated on an agarose or polyacrylamide gel.
- Run the samples. Real-time monitoring of migrating bands under a 254 nm low-pressure mercury lamp is possible.
- View or document the gel using available light source and an green/yellow filter. Transilluminators with blue light, or with UV low pressure mercury lamp (254 nm) can be used to visualize gels stained with ibGreen. High pressure mercury lamp (365 nm) can be used too, but this light source gives somewhat less efficient excitation.

Comparison between ethidium bromide and dsGreen

Feature	Ethidium Bromide	I05-G10000
Fluorescence	Red (615 nm)	Green (524 nm)
Excitation maximum	302 nm	454 nm
Excitation light source	UV only	Blue light - (UV less sensitive)
Sensitivity	2 ng / band (dsDNA) 100 ng / band (RNA)	0.08 ng / band (dsDNA) 1–2 ng / band (oligonucleotides)
Health hazard	High	Low

Loading Buffer II (6x)

Cat.-No.: i05-LB05 de 0,5 ml

For Acrylamide and Agarose Gels

Composition:

- Bromphenol blue sodium Salt: 0,25 %
- Xylene cyanol FF: 0,25 %
- Ficoll 400: 15 %

Storage:
The loading buffer is stable for 6 month at room temperature condition (RT)
For long term storage, more than 6 month please store at -20°C.

They increase the density of the sample and they add colour to the sample, thereby simplifying the loading process. They contain dyes that, in a electric field, move toward the anode at predictable rates. In 1% agarose gels, bromophenol blue migrates with 300 bp linear double-stranded DNA fragment, whereas xylene cyanol FF migrates at approximately the same rate as linear double-stranded DNA 4 kb length. These relationships are not significantly affected by the concentration (0.5 to 1.4%) of agarose in the gel. The gel – loading buffer contains only one low concentration dye (bromophenol blue and xylene cyanol FF) to avoid masking the DNA Ladder fragments. But if the added dye is masking your signal because it is running on the same high in your gel, just dilute it more.

How to predilute a DNA ladder with the loading dye:

For DNA markers, apply 0.1µg per 1mm of agarose gel lane width. Often 1µg of marker is used in one electrophoresis run but it depends on the size of your gel and the comb.

If DNA markers are not prediluted with the Loading dye solution, then mix :
The loading buffer is 6x concentrated, that means you have to use it 1:5.

DNA marker (IBIAN 1 kbp with 1µg/5µl) + 6X Loading Dye Solution + deionised water at a ratio 1:1:4

For example: 5µl 1 kb ladder + 5µl 6x loading dye + 20µl water.
By applying 30.0µl of this mixture, you'll have 1.0µg of total DNA per lane.

Order information

Catalog #	Pack size
i05-LB05	**0,5 ml**

IBIAN®-4you4 dNTP Mix (10 mM of each)

Cat.-No.: i02-M02 1000 µl

dNTPs are the building material for DNA molecule and used in various assays based on PCR. The purity of dNTPs is highly important for assay results' accuracy. The dNTPs synthesis itself doesn't except the presence of contaminants (such as NTPs, modified nucleotides, dNDPs, dNMPs, heavy/transition metals) in resulting solution, which can extremely affect the experiment by PCR inhibition. The use of a highly purified dNTP preparation is particularly recommended for sensitive techniques such as long-range PCR, RT-PCR, multiplex, mutagenesis experiments and Real-Time applications. HPLC is a suitable method of testing dNTP purity. dNTPs offered are HPLC tested and can be employed in highly sensitive assays.

The deoxynucleotides, PCR grade are suitable for many applications where high-quality reagents are required. Such procedures include reverse transcription (RT), polymerase chain reaction (PCR), RT-PCR, DNA labeling reactions and sequencing/cycle sequencing analysis.
All deoxynucleoside triphosphates are produced by the highly effective new chemical method developed in-house.

Description 4 you 4 dNTPs mix is a premixed solution (ready-to-use) in water of sodium salts of dATP, dCTP, dGTP and dTTP each at a concentration 10 mM. pH is adjusted to 7,5.

Purity assays:

- HPLC analysis (>98%); Not more then 5% of dNDPs were found by HPLC
- NMR analysis (inorganic phosphates) - passed
- Exo-endo deoxyribonucleases contamination test – passed
- UV-Spectral analysis – passed
- Spectophotometry – passed

Functional assays:

- Production of 8kb PCR fragment from genomic DNA with Taq DNA polymerase – passed
- Production of 0,6kb PCR fragment from genomic DNA with Pfu DNA polymerase - passed

Usage The solution is ready for use and is optimized for PCR. Use 1 microliter of 4 you 4 dNTPs mix in 50 microliters reaction volume (this equals a concentration of 200µM each dNTP).

Storage dNTPs can be stored at least 12 months at -20°C in a constant-temperature freezer. Avoid multiple freeze-thawing. For long-term usage, aliquoting is recommended.

Catalog #	Pack size
i02-M02	1000 µl
i02-M02X5	5 x 1000 µl

IBIAN®-Set of 4 dNTPs, 100 mM

Cat. N. i02-S11: **4 x 200 µl (100 mM each in 4 separate tube)**

Description: DNA molecules are built of dNTPs which are used in various PCR-based assays. The purity of dNTPs is highly important for the accuracy of assay results. The dNTPs synthesis itself doesn't except the presence of contaminants (such as NTPs, modified nucleotides, dNDPs, dNMPs, heavy/transition metals) in resulting solution, which can extremely affect the experiment by PCR inhibition. The use of a highly purified dNTP preparation is particularly recommended for sensitive techniques such as long-range PCR, RT-PCR, multiplex, mutagenesis experiments and Real-Time applications. HPLC is a suitable method to test dNTP purity. The offered dNTPs are HPLC tested and can be used in highly sensitive assays.
The set consists of 4 x 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP each supplied in a separate vial.
The deoxynucleotides are suitable for many applications where high-quality reagents are required. Such procedures include reverse transcription (RT), polymerase chain reaction (PCR), RT-PCR, DNA labeling reactions, and sequencing/cycle sequencing analysis.
All deoxynucleoside triphosphates are produced by an in-house developed highly effective new chemical method that is effectively used for a couple of years.

Solution: In water of sodium salts: 100 mM, pH 7.5

Purity assays:
HPLC analysis (> 98 %);
NMR analysis (inorganic phosphates) - passed
exo-endo deoxyribonucleases contamination test – passed
UV-spectral analysis – passed
spectophotometry – passed

Functional assays:
production of 8 kb PCR fragment from genomic DNA with Taq DNA polymerase – passed
production of 0.6 kb PCR fragment from genomic DNA with Pfu DNA polymerase - passed

dATP Na_4 * 3 H_2O
MW 634, 2'-Deoxyadenosine 5'-triphosphate, tetrasodium salt, Purity: 98.7 % (HPLC)

dCTP Na_4 * 3 H_2O
MW 609, 2'-Deoxycytidine 5'-triphosphate, tetrasodium salt, Purity: 98.9 % (HPLC)

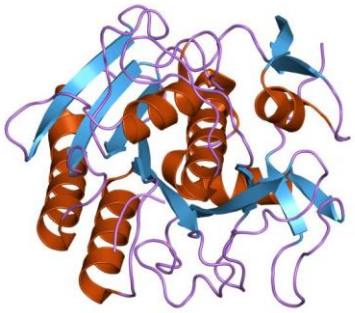
dGTP Na_4 * 3 H_2O
MW 649, 2'-Deoxyguanosine 5'-triphosphate, tetrasodium salt, Purity: 98.7 % (HPLC)

dTTP Na_4* 3 H_2O
MW 624, 2'-Desoxythymidine 5'-triphosphate, tetrasodium salt, Purity: 98.8 % (HPLC)

Catalog #	Pack size
i02-S11	4 x 200 µl
i02-S22	4 x 1000 µl

IBIAN®-Proteinase K Solution, 20 mg/ml

Description



Proteinase K is a non-specific serine protease with a very high specific activity. It has been used for isolation of mRNA, high molecular weight DNA and inactivation of other enzymatic activities. Proteinase K is active with or without the presence of SDS, EDTA and chaotropic salts.
Proteinase K is a broad-spectrum serine protease for general digestion of proteins in biological samples. The enzyme is free of RNase and DNase activities. The recommended working concentration for Proteinase K is 50 – 100 µg/mL in the majority of applications.

Shipping and storage conditions

Product is shipped at blue ice
Storage at -20°C. Proteinase K Solution is stable at RT for short time (up to 4 days). For long-term storage, we recommend -20 °C.

Content and order information

Catalog No.:	i06-PK01	i06-PK01x5
Proteinase K Solution 20 mg/ml	1 mL	5 x 1 mL
Datasheet	1	1

Liquid form (in 50 mM Tris-HCl pH 8.0, 1 mM CaCl2, 50 % glycerol)

Reaction buffer and specific activity

Reaction buffer: 50 mM Tris-HCl pH 7.5, 1 mM CaCl2
Specific activity: > 30 units/mg

Application:

Proteinase K Solution can be used for nucleic acid purification

Unit definition

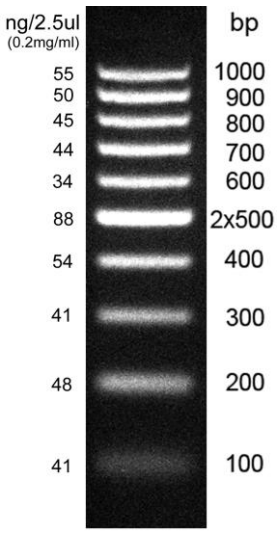
One unit is defined as the amount of enzyme that liberates Folin-positive amino acids and peptides, corresponding to 1 pmole tyrosine under assay conditions in 1 minute using haemoglobin as substrate

Quality control

- Digestion activity
- No detectable exo /endonuclease activity
- Absence of RNase contamination.

IBIAN®-100 bp DNA Ladder no stain 50 µg

Cat.-No.: i03-L05 50µg in 500 µl



Description: 100 bp DNA Ladder is ideal for determining the size of double-stranded DNA from 100 to 1,000 base pairs. The ladder consists of ten DNA fragments. Nine fragments are the products of the restrictions of special plasmids by EcoRV and/or RsaI restrictases. 100 bp fragment is PCR products. Pfu DNA polymerase was used for amplification. The 500 bp fragment is present at increased intensity to allow easy identification. All fragments are blunt-ended.

DNA fragments length (bp):

1000, 900, 800, 700, 2x500, 400, 300, 200, 100

Electrophoresis conditions:
Gel: 1% agarose
Buffer: 0.5x TBE
Voltage: 10 V/см

Concentration: 0.2 mg/ml

Storage buffer: 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM NaCl

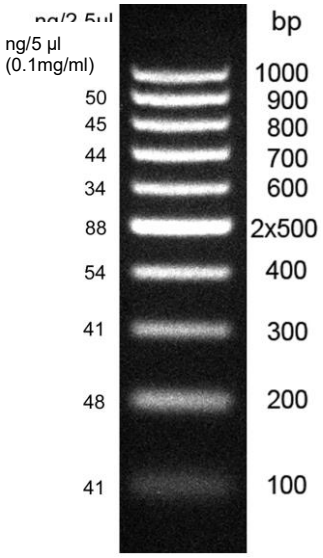
Storage: -20°C Product is shipped on blue-ice.

Usage recommendation: We recommend loading 0.5 µg of the DNA Ladder per line.

Catalog #	Pack size
i03-L05	0,05 mg (50µg)
i03-L05X5	0,25 mg (250µg)

IBIAN®-100 bp DNA Ladder Ready-to-use 50 µg

Cat.-No.: i03-B05 **50µg in 500µl**



Description: 100 bp DNA Ladder is ideal for determining the size of double-stranded DNA from 100 to 1,000 base pairs. The ladder consists of ten DNA fragments. Nine fragments are the products of the restrictions of special plasmids by EcoRV and/or RsaI restrictases. 100 bp fragment is PCR products. Pfu DNA polymerase was used for amplification. The 500 bp fragment is present at increased intensity to allow easy identification. All fragments are blunt-ended.

DNA fragments length (bp):

1000, 900, 800, 700, 2x500, 400, 300, 200, 100

Concentration: 0.1 mg/ml

Storage buffer: 10mM Tris-HCl, pH 7.5, 1 mM EDTA, 10mM NaCl, 6% glycerol, 0.05% Xylene Cyanol

Electrophoresis conditions:
Gel: 1% agarose
Buffer: 0.5x TBE
Voltage: 10 V/см

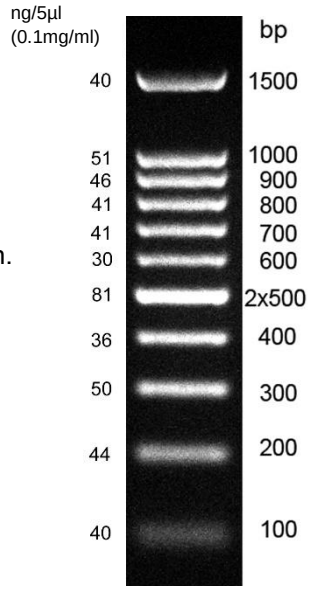
Storage: -20°C Product is shipped on blue-ice.

Usage recommendation: We recommend loading 5-6 µl of the DNA Ladder per line.

Catalog #	Pack size
i03-B05	0,05 mg (50µg)
i03-B055	0,25 mg (250µg)

IBIAN® 100 bp Plus DNA Ladder ready-to-use 50 µg

Ref. i03-B05P50µg in 500µl



Description: 100 bp plus ready-to-use DNA Ladder is ideal for determining the size of double-stranded DNA from 100 to 1,500 base pairs. The ladder consists of eleven DNA fragments. Ten fragments are the products of the restrictions of special plasmids by EcoRV and/or RsaI restrictases. 100 bp fragment is PCR products. Pfu DNA polymerase was used for amplification. The 500 bp fragment is present at increased intensity to allow easy identification. All fragments are blunt-ended. Recommended volume for loading is 5-6 µl per lane.

DNA fragments lenght (bp):

1500, 1000, 900, 800, 700, 600, 2x500, 400, 300, 200, 100

Concentration: 0.1 mg/ml

Storage buffer: 10mM Tris-HCl, pH 7.5, 1 mM EDTA, 10mM NaCl, 6% glycerol, 0.05% Xylene Cyanol

Storage: -20°C

Usage recommendation: We recommend loading 5-6 µl of the DNA Ladder per line.

Electrophoresis conditions:

Gel: 1% agarose

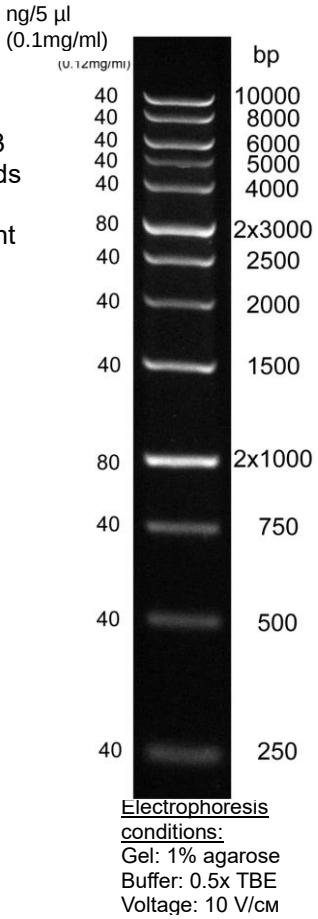
Buffer: 0.5x TBE

Voltage: 10 V/см

Catalog #	Pack size
i03-B05P	0,05 mg (50µg)
i03-B05PX5	0,25 mg (250µg)

IBIAN®-1 kb DNA Ladder no stain, 50 µg

Cat.-No.: i03-L10 **50µg in 500µl**



Electrophoresis conditions:
Gel: 1% agarose
Buffer: 0.5x TBE
Voltage: 10 V/см

Description: 1 kb DNA Ladder no stain is ideal for determining the size of double-stranded DNA from 250 to 10,000 base pairs. The ladder consists of 13 fragments. Ten fragments are the products of the restrictions of special plasmids by EcoRV and/or RsaI restrictases. 750, 500 and 250 bp fragments are PCR products. Pfu DNA polymerase was used for amplification. The 3.0 kb fragment and 1.0 kb fragment are present at increased intensity to allow easy identification. All fragments are blunt-ended.

DNA fragments lenght (kp):
10.0, 8.0, 6.0, 5.0, 4.0, 3.0x2, 2.5, 2.0, 1.5, 1.0x2, 0.75, 0.5, 0.25.

Concentration: 0.2 mg/ml

Storage buffer: 10mM Tris-HCl, pH 7.5, 1 mM EDTA, 10mM NaCl

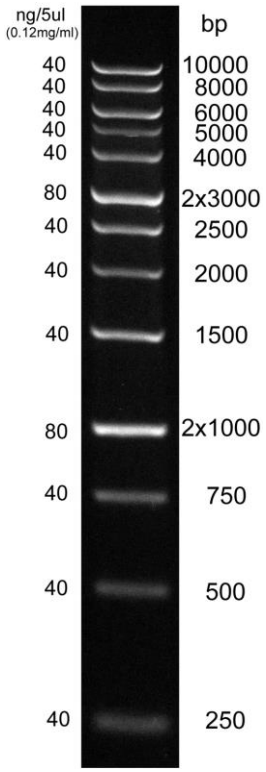
Storage: -20°C, product is shipped on blue-ice.

Usage recommendation: We recommend loading 0.5 µg of the DNA Ladder per line.

Catalog #	Pack size
i03-L10	0,05 mg (50µg)
i03-L10X5	0,25 mg (250µg)

IBIAN®-1 kb DNA Ladder Ready-to-use, 50 µg

Cat.-No.: i03-B10 50µg in 500µl



Description: 1 kb DNA Ladder ready-to-use is ideal for determining the size of double-stranded DNA from 250 to 10,000 base pairs. The ladder consists of 13 fragments. Ten fragments are the products of the restrictions of special plasmids by EcoRV and/or RsaI restrictases. 750, 500 and 250 bp fragments are PCR products. Pfu DNA polymerase was used for amplification. The 3.0 kb fragment and 1.0 kb fragment are present at increased intensity to allow easy identification. All fragments are blunt-ended. Recommended volume for loading is 5-6 ul per line.

DNA fragments lenght (kp):
10.0, 8.0, 6.0, 5.0, 4.0, 3.0x2, 2.5, 2.0, 1.5, 1.0x2, 0.75, 0.5, 0.25.

Concentration: 0.1 mg/ml

Storage buffer: 10mM Tris-HCl, pH 7.5, 1 mM EDTA, 10mM NaCl, 6% glycerol, 0,05% Xylene Cyanol

Storage: -20°C, product is shipped on blue-ice.

Electrophoresis conditions:
Gel: 1% agarose
Buffer: 0.5x TBE
Voltage: 10 V/см

Usage recommendation: We recommend loading 5-6 µl of the DNA Ladder per line (0.5 µg of the DNA Ladder)

Catalog #	Pack size
i03-B10	0,05 mg (50µg)
i03-B105	0,25 mg (250µg)