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green line DATA SHEET

> XtraTaq Pol RTL GL (ready to load - Green Line)

cat. no.	amount	note
XSTS-T5XRTL 250 GL	250 units	5X XtraRTL Buffer GL
XSTS-T5RTL 1000 GL	1000 units	5X XtraRTL Buffer GL
XSTS-T5XRTLw 250 GL	250 units	5X XtraRTL Buffer GL w/o MgCl2
XSTS-T5XRTLw 1000 GL	1000units	5X XraRTL Buffer GL w/o MgCl2

add nucleotide box

cat. no.	amount	note
XSTSn-T5XRTL 250 GL	250 units	5X XtraRTL Buffer GL + dNTPs
XSTSn-T5XRTL 1000 GL	1000 units	5X XtraRTL Buffer GL + dNTPs
XSTSn-T5XRTLw 250 GL	250 units	5X XtraRTL Buffer GL w/o MgCl2 + dNTPs
XSTSn-T5XRTLw 1000 GL	1000units	5X XtraRTL Buffer GL w/o MgCl2 + dNTPs

An highly processive, recombinant (from E.coli strain), thermostable DNA with a very high efficiency of 5'- 3' polymerase activity and 3´- 5' exonuclease (non-proofreading) activity. Xtra Taq Pol catalyzes the addition of mononucleotide units to the 3'-end of a primer chain, leading to the formation of DNA products that have 3'-overhanging A nucleotides (thus can be used in TA cloning). This enzyme remains funtional even after prolonged incubation steps at 95°C. The enzyme is supplied at 5U/µl and comes with 5X XtraRTL GL (Ready To Load) new buffer. 5X XtraRTL GL Reaction Buffer is a Genespin proprietary formulation, developed for standard and/or high-fidelity amplification of high-GC (>75%) templates. The buffer contain 7.5mM magnesium, PCR enhancers and thickening agents (vortex thoroughly prior to use). **5X XtraRTL GL contain an internal fluorescent stain for DNA detection on Agarose gel directly after PCR amplification.** 5X XtraRTL GL Reaction Buffer contains Orange G dye that allows gel loading and electrophoresis of the sample directly from the PCR tube, without further manipulation. The Orange G dye migrates at the same rate as a duplex DNA fragment of approximately 50 Kbp and does not interfere with DNA migration when it is used as a loading dye for agarose gel electrophoresis.

FOR RESEARCH USE ONLY

UNIT DEFINITION
One unit is defined as the amount of enzyme required to incorporate 10 nanomoles of dNTPs into acid-insolubile material in 30 min at 74°C.

SHIPPING
Shipped in green ice.

STORAGE
Store at -20C°

SHELF LIFE
12 months

FORM
Liquid

CONCENTRATION
5U/ul

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component	STS-T5XRTL 250 GL	STS-T5XRTLw250 GL	STSn-T5XRTL250 GL	STSn-T5XRTLw250 GL
Xtra Taq Polymerase	250 units / 50ul	250 units / 50ul	250 units / 50ul	250 units / 50ul
Standard Buffer	1.5ml 5X XtraRTL Buffer GL with MgCl2	1.5ml 5X XtraRTL Buffer GL w/o MgCl2MgCl2	1.5ml 5X XtraRTL Buffer GL with MgCl2MgCl2	1.5ml 5X XtraRTL Buffer GL w/o MgCl2Mg
MgCl2	-	500ul / 50mM	-	500ul / 50mM
dNTPs	-	-	100ul / 10mM each	100ul / 10mM each

component	STS-T5XRTL1000 GL	STS-T5XRTLw1000 GL	STSn-T5XRTL1000 GL	STSn-T5XRTLw1000 GL
Xtra Taq Polymerase	*1000* units / 200ul	*1000* units / 200ul	*1000* units / 200ul	*1000* units / 200ul
Standard Buffer	4ml 5X XtraRTL Buffer GL with MgCl2MgCl2	4ml 5X XtraRTL Buffer GL w/o MgCl2MgCl2MgCl2	4ml 5X XtraRTL Buffer GL with MgCl2MgCl2	4ml 5X XtraRTL Buffer GL w/o MgCl2MgCl2MgCl2
MgCl2	-	500ul / 50mM	-	500ul / 50mM
dNTPs	-	-	400ul / 10mM each	400ul / 10mM each

Assay Set-Up:

Before starting, vortex all components thoroughly to ensure homogeneity.
Prepare a premix for the number of assays you need according to the following protocol:

component	stock conc.	final conc.	30ul reaction
5X Buffer GL	5X	1X	6.0ul
dNTPs	10mM each.	200uM	0.6ul
Xtra Taq Polymerase	5U/ul	0.025U/ull	0.2ul
primers	1ug/ul each	50ng/ul each	2ul each
DNA Template	-	-	10-20ng
MG Water	-	-	up to 30ul

Cycling conditions:

Spin down the tubes/plate briefly to remove bubbles and place them into the cycler.

denaturation	95°C	5 min	1x
denaturation	95°C	30 sec	20-35x
annealing (1)	50-68°C	30 sec	
extension (2)	72°C	30sec	

1)The annealing temperature depends on the melting temperature of the primers used.
2)The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

new molecular biology

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OVERVIEW

Scientist to Scientist

GeneSpin PCR green line

green line

No more post or pre-staining protocol for DNA on Agarose Gel

GeneSpin PCR geen line (Genespin proprietary formulation), is a specific PCR products line focused on **users safety.**
Either 2X PCR Mastermixes GL or 5x PCR buffers GL contain an internal fluorescent stain for DNA detetion on Agarose gel directly after PCR amplification. This particular composition is able to avoid standard protocols for post- or pre- staining DNA on Agarose Gel with Ethidium Bromide (EtBr) or different dsDNA stains. The flouorescent used in **GeneSpin PCR geen line regents** has higher sensitivity than EtBr and has an easy, fast and robust staining procedure. Detection is possible by illuminating the Agarose Gel on a UV screen. Ames test II has shown a lower mutagenic potential compared to SYBR Green I and a much lower mutagenic potential than EtBr. Storage: protect GeneSpin GL reagents from light.

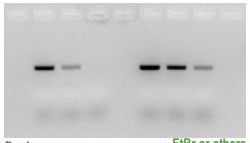


fig 1a - EtBr or others

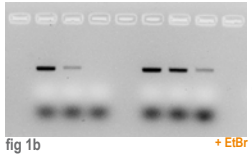


fig 1b + EtBr

Fig. 1: Comparisons of the amplification efficiency of ***new 2X XtraRTL Master Mix GL (fig.1a) versus 2X XtraRTL Master Mix (fig.1b)*** were performed using serial dilutions (1:1, 1:10, 1:100) of rattus cDNA and specific primers corresponding to beta-actin. New ***2X XtraRTL Master Mix GL*** shows the same efficiency in amplifying the beta-actin region than ***2X XtraRTL Master Mix*** . Detection of ***2X XtraRTL Master Mix GL*** PCR product is performed without post- or pre-staining by illuminating the agarose gel (3%) on a UV screen (fig 1a).

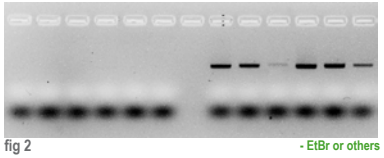


fig 2 - EtBr or others

Fig. 2: Detection of ***2X XtraRTL Master Mix GL*** PCR product versus ***2X XtraRTL Master Mix*** PCR product is performed without post- or pre-staining on 3% agarose gel (fig 2) by illuminating the agarose gel on a UV screen. Only ***2X XtraRTL Master Mix GL*** PCR product is visible on the agarose gel.Both Mastermixes are visible on 3% agarose gel after pre- or post-staining with EtBr (data not shown).The amplification was performed using serial dilutions (1:1, 1:10, 1:100) of two different U2OS human cells INPUT DNA and specific primers corresponding to a centromeric region (SatCen11).

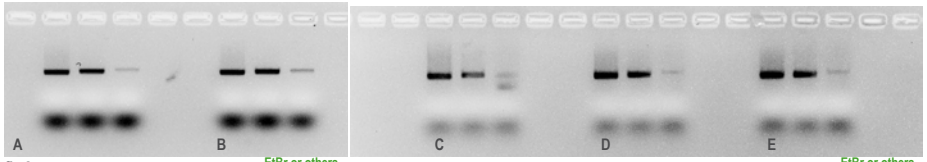


fig 3 - EtBr or others - EtBr or others

Fig. 3: Detection of ***GeneSpin PCR green line products*** on 3% agarose gel (fig 3) by illuminating the agarose gel on a UV screen without post- or pre-staining. The PCR amplification was performed using serial dilutions (1:1, 1:10, 1:100) of U2OS human cells INPUT DNA and specific primers corresponding to SON region. ***2X XtraRTL Master Mix GL (fig. 3.a), Xtra Taq (5U/ul) + 5X Xtra RTL Buffer GL (fig. 3.b), GS Taq Pol +10X PCR Buffer (fig. 3.c), 2X XtraWhite Master Mix GL (fig. 3.d), Xtra Taq (5U/ul) + 5X Xtra White Buffer GL (fig. 3.e).***

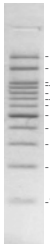


fig 4a

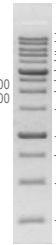


fig 4b

Fig. 4: ***GeneSpin 100bp GL*** (fig 4.a) ***and 1kb GL ladders*** (fig 4.b) were optimized for direct loading onto unstained agarose gels.
The ladders provide highest level of convenience during the routine handling and avoid commonly used gel staining procedures with ethidium bromide or SYBR Green I.

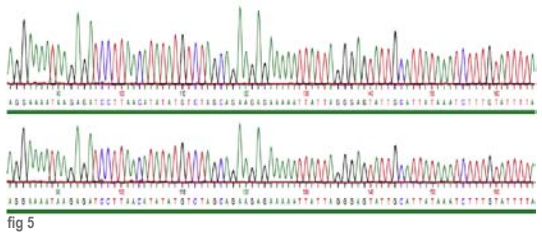
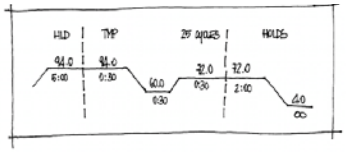


fig 5

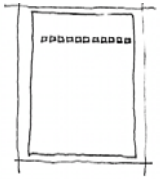
Fig. 5: Sequence analysis from PCR products of both ***2X XtraRTL Master Mix*** (up) and ***2X XtraRTL Master Mix GL*** (down).

1. PCR* Sample Preparation

2. Run PCR*



3. Run Agarose Gel



4. Check under UV ligh

use UV light to detect signal

without add Gel Stain (NO EtBr or others)

ORDERING Information

CAT#: STS-XMMixW GL1000 **2X XtraWhite Master Mix GL** (1000 reactions - 50ul) - 25ml
CAT#: STS-XMMixW GL200 **2X XtraWhite Master Mix** GL (200 reactions - 50ul) - 5ml
CAT#: STS-XMMixRTL GL1000 **2X XtraRTL Master Mix GL** (1000 reactions - 50ul) - 25ml
CAT#: STS-XMMixRTL GL200 **2X XtraRTL Master Mix GL** (200 reactions - 50ul) - 5ml

CAT#: STS-HXMMixW GL1000 **2X Hot StartXtraWhite Master Mix GL** (1000 reactions - 50ul) - 25ml
CAT#: STS-HXMMixW GL200 **2X Hot StartXtraWhite Master Mix** GL (200 reactions - 50ul) - 5ml
CAT#: STS-HXMMixRTL GL1000 **2X Hot StartXtraRTL Master Mix GL** (1000 reactions - 50ul) - 25ml
CAT#: STS-HXMMixRTL GL200 **2X Hot StartXtraRTL Master Mix GL** (200 reactions - 50ul) - 5ml

CAT#: XSTS-T5XRTL GL1000 **XtraTaq Pol with 5X XtraRTL GL Buffer** (1000U)
CAT#: XSTS-T5XRTL GL250 **XtraTaq Pol with 5X XtraRTL GL Buffer** (250U)
CAT#: XSTS-T5XW GL1000 **XtraTaq Pol with 5X XtraWhite GL Buffer** (1000U)
CAT#: XSTS-T5XW GL250 **XtraTaq Pol with 5X XtraWhite GL Buffer** (250U)

CAT#: STS-T1000 GL **Taq Pol with 10X Reaction Buffer GL** (1000U)
CAT#: STS-T250 GL **Taq Pol with 10X Reaction Buffer GL** (250U)

CAT#: STS-1Kb GL **DNA Ladder GL 1Kb**
CAT#: STS-100bp GL **DNA Ladder GL 100bp**

*For research only, not for resale

**The PCR process, which is the subject of European Pat. Nos. 201,184 and 200,362 owned by Hoffmann-La Roche*, is covered by patents issued and applicable in certain countries. GeneSpin does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.
* The above primary European Pat. Nos. 201,184 and 200,362 expired on March 28, 2006.
In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

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