



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 MgCl ₂ |
| XSTS-T5XRTLw 1000 GL | 1000units | 5X XtraRTL Buffer GL w/o MgCl ₂ |

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 MgCl ₂ + dNTPs |
| XSTSn-T5XRTLw 1000 GL | 1000units | 5X XtraRTL Buffer GL w/o MgCl ₂ + 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 functional 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-insoluble 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

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

| 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 MgCl ₂ | 1.5ml 5X XtraRTL Buffer GL w/o MgCl ₂ MgCl ₂ | 1.5ml 5X XtraRTL Buffer GL with MgCl ₂ MgCl ₂ | 1.5ml 5X XtraRTL Buffer GL w/o MgCl ₂ Mg |
| MgCl ₂ | - | 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 MgCl ₂ MgCl ₂ | 4ml 5X XtraRTL Buffer GL w/o MgCl ₂ MgCl ₂ MgCl ₂ | 4ml 5X XtraRTL Buffer GL with MgCl ₂ MgCl ₂ | 4ml 5X XtraRTL Buffer GL w/o MgCl ₂ MgCl ₂ MgCl ₂ |
| MgCl ₂ | - | 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.



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

GeneSpin PCR green 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 detection 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 fluorescent stain used in **GeneSpin PCR green line reagents** 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.

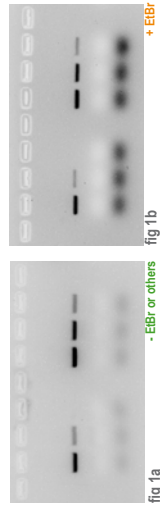


Fig. 1: Comparisons of the amplification efficiency of **new 2X XtraRTL Master Mix GL (fig. 1a) versus 2X XtraRTL Master Mix GL (fig. 1b)** were performed using serial dilutions (1:1, 1:10, 1:100) of rattus cDNA and specific primers corresponding to beta-actin. **New 2X XtraRTL Master Mix GL** shows the same efficiency in amplifying the beta-actin region than **2X XtraRTL Master Mix GL**. 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).

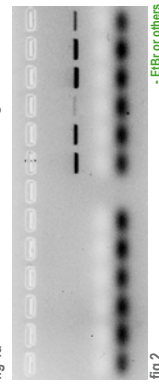


Fig. 2: Deletion of **2X XtraRTL Master Mix GL** PCR product, versus **2X XtraRTL Master Mix GL** PCR product, is performed, without post- or pre-staining on 3% agarose gel (fig. 2) by illuminating the agarose gel on a UV screen. Only **2X XtraRTL Master Mix GL** PCR product is visible on the agarose 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 INPUTDNA and specific primers corresponding to a centromeric region (SatCent11).

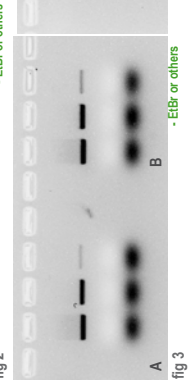


Fig. 3: Detection of **GeneSpin PCR green line products** on 3% agarose gel (fig. 3) by illuminating the agarose gel on a UV screen without post- or pre-staining. The PCR amplification was performed using serial dilutions (1:1, 1:10, 1:100) of U2OS human cells INPUT DNA and specific primers corresponding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

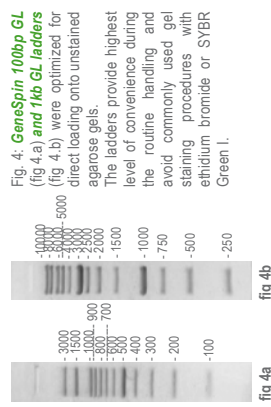


Fig. 4: **GeneSpin 100bp GL (fig. 4.a)** and **1kb GL ladders (fig. 4.b)** were optimized for direct loading onto unstained agarose gels. The ladders provide highest level of convenience during the routine handling and avoid commonly used gel staining procedures with ethidium bromide or SYBR Green I.

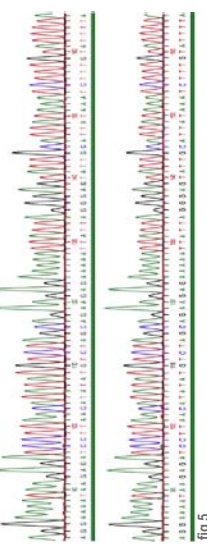
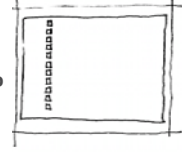


Fig. 5: Sequence analysis from PCR products of both **2X XtraRTL Master Mix GL (up)** and **2X XtraRTL Master Mix GL (down)**.

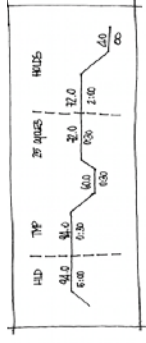
1. PCR* Sample Preparation



3. Run Agarose Gel



2. Run PCR*



4. Check under UV light



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-15XRTL GL1000 XtraTaq Pol with 5X XtraRTL GL Buffer (1000U)
- CAT#: XSTS-15XRTL GL250 XtraTaq Pol with 5X XtraRTL GL Buffer (250U)
- CAT#: XSTS-15XW GL1000 XtraTaq Pol with 5X XtraWhite GL Buffer (1000U)
- CAT#: XSTS-15XW 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.