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## green line DATA SHEET

### > 2X Master Mix Standard GL (Green Line)

cat. no.	amount	note
STS-XMMixW 200* GL	5ml	2X XtraWhite Master Mix GL
STS-XMMixW 1000* GL	25ml	2X XtraWhite Master Mix GL
STS-XMMixRTL 200 GL	5ml	2X XtraRTL Master Mix GL
STS-XMMixRTL 1000 GL	25ml	2X XtraRTL Master Mix GL

\*2X XtraWhite Master Mix is supplied with appropriate quantity of 6X Loading Dye

2X XtraWhite Master Mix GL and 2X XtraRTL Master Mix GL (Genespin proprietary formulation), are two premixed, ready-to-use solution containing Xtra-Taq Pol, dNTPs and MgCl<sub>2</sub> in a Reaction Buffer optimized for use in PCR amplification of targets present in low copy number and to avoid amplification of non-specific products. Both buffers contain 3.0mM magnesium, PCR enhancers and thickening agents (vortex thoroughly prior to use). **2X Master Mix Standard GL contain an internal fluorescent stain for DNA detection on Agarose gel directly after PCR amplification.** 2X XtraRTL Master Mix GL may contains Orange G dye or a combination of tartrazine and xylene dyes that allow gel loading and electrophoresis of the sample directly from the PCR tube, without further manipulation. 2X XtraWhite Master Mix GL is supplied with appropriate quantity of 6X Loading Dye. The dyes migrate at the same rate as a duplex DNA fragment of approximately from 40-50 bp to 4160 bp and do not interfere with DNA migration when they are used as a loading dye for agarose gel electrophoresis.

#### FOR RESEARCH USE ONLY

##### SHIPPING

Shipped in green ice.

##### STORAGE

Store at -20C°. Avoid freeze/thaw cycles.

##### SHELF LIFE

12 months

##### FORM

Liquid

##### CONCENTRATION

2X conc.



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#### 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
2X Master Mix GL	2X	1X	15.0ul
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.

# Scientist to Scientist GeneSpin PCR 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 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.

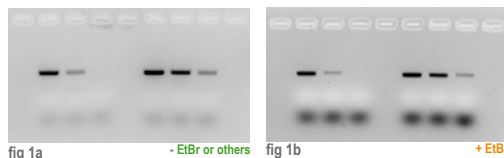


Fig. 1: Comparisons of the amplification efficiency of **new 2X XtraRTL Master Mix GL (fig. 1a)** versus **2X XtraRTL Master Mix (fig. 1b)** were performed using serial dilutions (1:1, 1:10, 1:100) of *rattus* cDNA and specific primers corresponding to beta-actin. **New 2X XtraRTL Master Mix GL** shows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

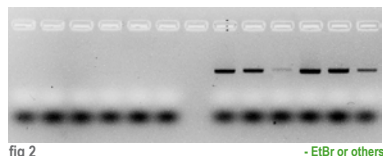


Fig. 2: Detection of **2X XtraRTL Master Mix GL** PCR product versus **2X XtraRTL Master Mix** PCR product is performed without post- or pre-staining on 3% agarose gel (fig 2) by illuminating the agarose gel on a UV screen. Only **2X XtraRTL Master Mix GL** PCR product is visible on the agarose gel. 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).

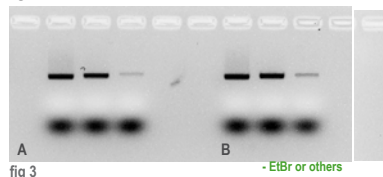


Fig. 3: Detection of **GeneSpin PCR green line products** on 3% agarose gel (fig 3) by illuminating the agarose gel on a UV screen without post- or pre-staining. The PCR amplification was performed using serial dilutions (1:1, 1:10, 1:100) of U2OS human cells INPUT DNA and specific primers corresponding 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)**.

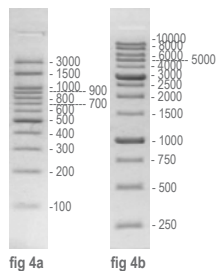


Fig. 4: **GeneSpin 100bp GL (fig 4.a)** and **1kb GL ladders (fig 4.b)** were optimized for direct loading onto unstained agarose gels. The ladders provide highest level of convenience during the routine handling and avoid commonly used gel staining procedures with ethidium bromide or SYBR Green I.

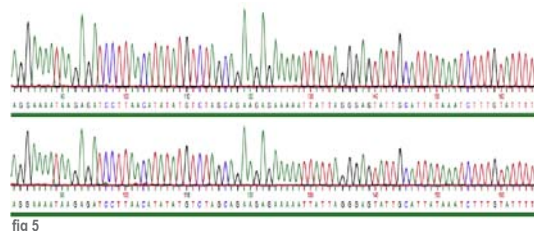
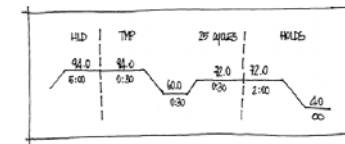


Fig. 5: Sequence analysis from PCR products of both **2X XtraRTL Master Mix GL (up)** and **2X XtraRTL Master Mix GL (down)**.

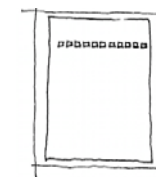
### 1. PCR\* Sample Preparation



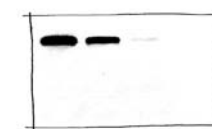
### 2. Run PCR\*



### 3. Run Agarose Gel



### 4. Check under UV light



without add Gel Stain (NO EtBr or others)

use UV light to detect signal

## 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.