

Certificate of Analysis & Product Manual



Triple Repeat Disorders Genotyping
Fragile X, Myotonic Dystrophy, Friedreich's Ataxia, Huntington's disease, SCA2, SCA3, SBMA, DRPLA
Fluorescent Probes, siRNA, Hybridization and Detection Reagents

SCA2 GScan™ Ver2 Kits

Spinocerebellar Ataxia Type 2 (SCA2) ATXN2 Gene CAG Repeats Genotyping

Catalog No. 40-2038-15

Storage Condition: See Material Supplied List

For Research Use Only. Not for use in diagnostic procedures for clinical purposes

Important Information

SCA2 GScan™ Version 2 kit components are not compatible with earlier version kit components.

All Gene Link products are for research use only.

Not for use in diagnostic procedures for clinical purposes.

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument and software capable of detection of fluorescently labeled fragments of varying lengths.

Product to be used by experienced researchers appropriately trained in performing molecular biology techniques following established safety procedures. Additional qualification and certification is required for interpretation of results.



Material Supplied

SCA2 GScan™ Ver2 Kits

Spinocerebellar Ataxia Type 2 (SCA2) CAG Repeats Genotyping

SCA 2 GScan™ Version 2 kit components are not compatible with earlier version kit components.

SCA2 (ATXN2) CAG Repeat Genotyping GScan™ V2 Kits

| Kit | Catalog No. | SCA2 GScan™ V2 Kits 100 X 25 µL Reactions | Size |
|--------------------------|---------------|--|----------|
| <input type="checkbox"/> | 40-2038-15FM | SCA2 (ATXN2) CAG Repeat Genotyping GScan™ V2 6-Fam Kit, 100 rxns | 100 rxns |
| | | SCA2 GScan™ V2 Kits 20 X 25 µL Reactions | |
| <input type="checkbox"/> | 40-2038-15FMS | SCA2 (ATXN2) CAG Repeat Genotyping GScan™ V2 6-Fam Kit, 20 rxns | 20 rxns |

SCA2 (ATXN2) CAG Repeat Genotyping GScan™ V2 Kit 100 Reactions Components

| Content | Catalog No. | SCA2 GScan™ V2 Kits 100 X 25 µL Reactions | Size |
|--------------------------|---------------|---|--------|
| <input type="checkbox"/> | 40-2038-15AFM | SCA2 GScan™ V2 6-Fam Component A | 600 µL |
| <input type="checkbox"/> | 40-2038-15E | SCA2 GScan™ V2 Component E | 800 µL |
| <input type="checkbox"/> | 40-2038-15M | SCA2 GScan™ V2 Component M | 450 µL |
| <input type="checkbox"/> | 40-2038-01S | SCA2 GScan™ Control Genemer™ DNA 22 Repeats (19 CAG+2 CAA repeats) control. ~1 ng/ µL | 25 µL |

SCA2 (ATXN2) CAG Repeat Genotyping GScan™ V2 Kit 20 Reactions Components

| Content | Catalog No. | SCA2 GScan™ V2 Kits 100 X 25 µL Reactions | Size |
|--------------------------|----------------|---|--------|
| <input type="checkbox"/> | 40-2038-15AFMS | SCA2 GScan™ V2 6-Fam Component F | 140 µL |
| <input type="checkbox"/> | 40-2038-15ES | SCA2 GScan™ V2 Component E | 175 µL |
| <input type="checkbox"/> | 40-2038-15MS | SCA2 GScan™ V2 Component M | 100 µL |
| <input type="checkbox"/> | 40-2038-01SS | SCA2 GScan™ Control Genemer™ DNA 22 Repeats (19 CAG+2 CAA repeats) control. ~1 ng/ µL | 10 µL |

Storage Condition: Store at -20°C

Certificate of Analysis & Product Specifications


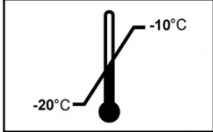

The SCA2 GScan™ V2 kit components supplied has been validated to amplify the CAG triple repeat spanning region of the ATXN2 gene. The length of CAG triple repeat amplification routinely obtained by using standard Taq polymerase is greater than 120 CAG repeats.




Appropriate nuclease free handling, dispensing and storage conditions required.

Manufacturing lot numbers are stated on the label of each product and accompanying packing slip.

Product Label Information

| REF | Catalog No. | Description | Size |
|-----|---------------|---|----------|
| | 40-2038-15FM | SCA2 (ATXN) CAG Repeat Genotyping GScan™ V2 6-Fam Kit, 100 rxns | 100 rxns |
| | 40-2038-15FMS | SCA2 (ATXN) CAG Repeat Genotyping GScan™ V2 6-Fam Kit, 20 rxns | 20 rxns |

| | | |
|---|---|---|
|  |  |  |
| Research Use Only | Storage Store at -20°C to -10°C | Lot Number Stated on product tube and packing slip |

| | | |
|---|---|---|
|  |  |  |
| Expiry One year from Date of Shipment | Instructions Consult product manual | QR Code Visit Gene Link website for product details |

Spinocerebellar Ataxia Type 2 (SCA2) Genotyping

Background

The autosomal dominant cerebellar ataxias (ADCA) are a heterogeneous group of neurodegenerative disorders characterized by progressive degeneration of the cerebellum, brain stem and spinal cord. Spinocerebellar ataxia (SCA) type 2 is characterized by deterioration in balance and coordination, slow saccadic eye movement, and in some individuals ophthalmoparesis.

People with this condition initially experience problems with coordination and balance (ataxia). Other early signs and symptoms of SCA2 include speech and swallowing difficulties, rigidity, tremors, and weakness in the muscles that control eye movement (ophthalmoplegia). Eye muscle weakness leads to a decreased ability to make rapid eye movements (saccadic slowing).

Over time, individuals with SCA2 may develop loss of sensation and weakness in the limbs (peripheral neuropathy), muscle wasting (atrophy), uncontrolled muscle tensing (dystonia), and involuntary jerking movements (chorea). Individuals with SCA2 may have problems with short term memory, planning, and problem solving, or experience an overall decline in intellectual function (dementia).

Signs and symptoms of the disorder typically begin in mid-adulthood but can appear anytime from childhood to late adulthood. People with SCA2 usually survive 10 to 20 years after symptoms first appear. SCA2 is inherited in an autosomal dominant manner. Offspring of an affected individual have a 50% chance of inheriting the gene mutation.

Mutations in the *ATXN2* gene cause SCA2. The *ATXN2* gene provides instructions for making a protein called ataxin-2. This protein is found throughout the body, but its function is unknown. Ataxin-2 is found in the fluid inside cells (cytoplasm), where it appears to interact with a cell structure called the endoplasmic reticulum. The endoplasmic reticulum is involved in protein production, processing, and transport. Researchers believe that ataxin-2 may be involved in processing RNA, a chemical cousin of DNA. Ataxin-2 is also thought to play a role in the production of proteins from RNA (translation of DNA's genetic information).

The *ATXN2* gene mutations that cause SCA2 involves a DNA segment known as a CAG trinucleotide repeat. This segment is made up of a series of three DNA building blocks (cytosine, adenine, and guanine) that appear multiple times in a row. Normally, the CAG segment is repeated approximately 22 times within the gene, but it can be repeated up to 31 times without causing any health problems. Individuals with 32 or more CAG repeats in the *ATXN2* gene develop SCA2. People with 32 or 33 repeats tend to first experience signs and symptoms of SCA2 in late adulthood, while people with more than 45 repeats usually have signs and symptoms by their teens.

It is unclear how the abnormally long CAG segment affects the function of the ataxin-2 protein. The abnormal protein apparently leads to cell death, as people with SCA2 show loss of brain cells in different parts of the brain. Over time, the loss of brain cells causes the movement problems characteristic of SCA2.

The mutation in all identified SCA genes is the expansion of an unstable CAG repeat encoding a polyglutamine tract. Similar to other trinucleotide repeat disorders, such as Huntington disease and spinal and bulbar muscular atrophy, the SCAs show anticipation and different degrees of expansion in maternal or paternal transmission. There is a direct correlation between the size of the CAG repeat and the onset and severity of the disease. Affected adult

individuals have alleles with 36-64 CAG trinucleotide repeats, while infantile- and juvenile-onset SCA2 is associated with expansions of 130 to more than 200 CAG trinucleotide repeats.

The SCA2 locus has been mapped to chromosome 12q24. Several SCA genes have been cloned and shown to contain CAG repeats in their coding regions. Spinocerebellar ataxia (SCA) 1, 2, 3, 4 and 6, are assigned to five different chromosomes.

Table 1. Trinucleotide Repeats in Human Genetic Disease

| Disease | Repeat ^a | Normal Length ^b | Intermediate Length (Premutation) ^{a,b} | Full Disease Length ^b |
|--|---------------------|----------------------------|--|-----------------------------------|
| Fragile XA (FRAXA) | (CGG) _n | 6-52 | 59-230 | 230-2,000 |
| Fragile XE (FRAXE) | (CCG) _n | 4-39 | ? (31-61) | 200-900 |
| Fragile XF (FRAXF) | (CGG) _n | 7-40 | ? | 306-1,008 |
| FRA16A | (CCG) _n | 16-49 | ? | 1,000-1,900 |
| Jacobsen Syndrome (FRA11B) | (CGC) _n | 11 | 80 | 100-1,000 |
| Kennedy Syndrome (SMBA) | (CAG) _n | 14-32 | ? | 40-55 |
| Myotonic Dystrophy (DM) | (CTG) _n | 5-37 | 50-80 | 80-1,000; congenital, 2,000-3,000 |
| Huntington disease (HD) | (CAG) _n | 10-34 | 36-39 | 40-121 |
| Spinocerebellar ataxia 1 (SCA1) | (CAG) _n | 6-39 | None Reported | 40-81 |
| Spinocerebellar ataxia 2 (SCA2) | (CAG) _n | 14-31 | None Reported | 34-59 |
| Spinocerebellar ataxia 3 (SCA3)/Machado Joseph disease (MJD) | (CAG) _n | 13-44 | None Reported | 60-84 |
| Spinocerebellar ataxia 6 (SCA6) | (CAG) _n | 4-18 | None Reported | 21-28 |
| Spinocerebellar ataxia 7 (SCA7) | (CAG) _n | 7-17 | 28-35 | 38-130 |
| Haw River syndrome (HRS; also DRPLA)) | (CAG) _n | 7-25 | ? | 49-75 |
| Friedreich ataxia (FRDA) | (GAA) _n | 6-29 | ? (>34-40) | 200-900 |

^a Typically, repeats tracts contain sequence interruptions. See Pearson and Sinden (1998a) for a discussion of the sequence interruptions.

^b No. of triplet repeats.

^c A question mark (?) indicates potential mutagenic intermediate length, and an ellipsis (...) indicates none. Not all diseases are associated with a premutation length repeats tract or premutation disease condition.

Molecular Analysis

ATXN2 is the only gene known to be associated with SCA2. One hundred percent of individuals affected with SCA2 have a CAG trinucleotide repeat expansion. The presence of one abnormal allele is diagnostic. Normal alleles CAG repeats are below 31. DNA analysis can detect 100% of expanded alleles.

SCA2 genotyping can be done by direct PCR amplification of the CAG trinucleotide repeats region or by southern analysis. In most cases both methods are used to complement the results.

Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods. PCR-Prober™, GScan™ and GeneProber™ line of products replaces radioactive based methods. Gene Link's GScan Ver2 kit is for PCR amplification followed by fluorescent detection of the specific triple repeat fragment size and routinely detects greater than 120 CAG repeats.

Genemer™ Kit Agarose Gel Analysis

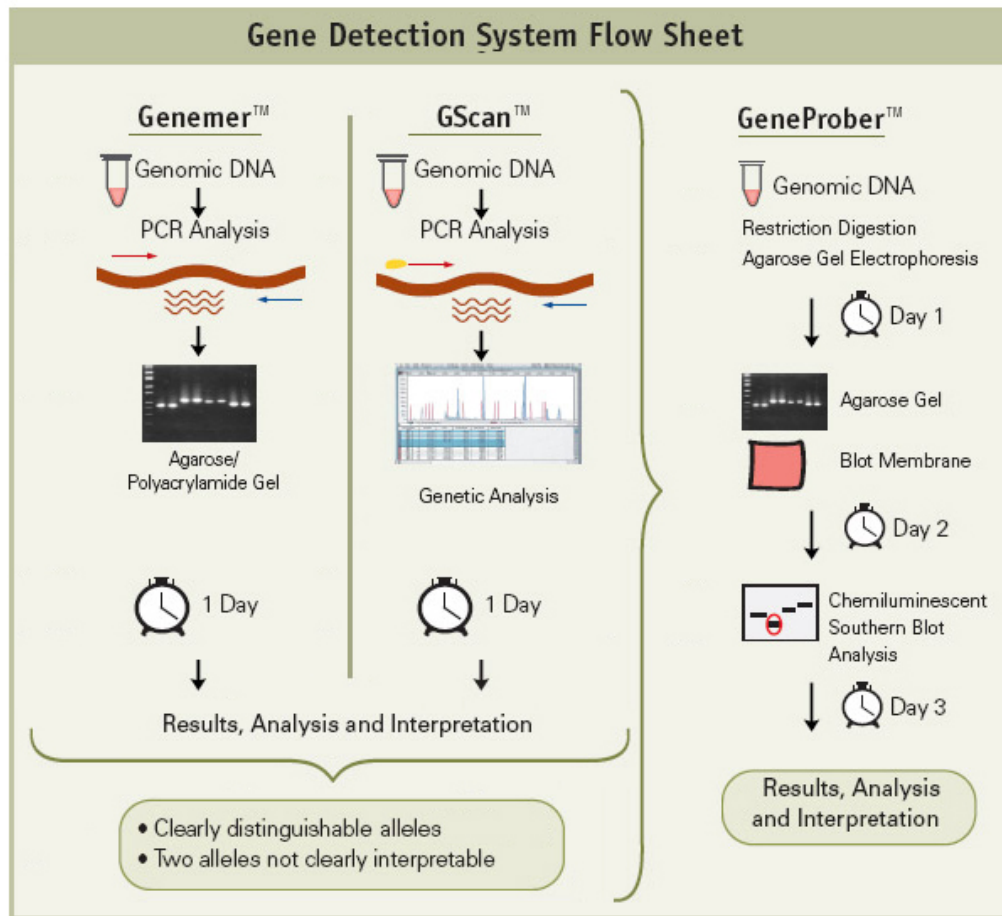
Optimized Genemer™ kit with components for PCR amplification of up to 130 Fragile X CGG repeats using standard Taq polymerase. Amplified samples are resolved by agarose gel electrophoresis. This Genemer™ method or GScan™ fluorescent detection is recommended for initial screening of all samples.

GScan™ Kit

Optimized GScan™ kit with components for PCR amplification of up to 130 Fragile X CGG repeats using standard Taq polymerase. Amplified samples are resolved by genetic analyzers capable of fluorescent detection or agarose gel electrophoresis. This Genemer™ Kit or GScan™ kit for fluorescent detection is recommended for initial screening of all samples.

GeneProber™ Probes for Southern Blot Analysis

Digoxigenin labelled probes for chemiluminescent Southern blot detection method or unlabeled probe for end user to perform radioactive label. Gene Link offers safe and reliable chemiluminescent detection methods as an alternate to radioactive based detection methods.



| SCA 2 CAG Fragment A Expected Length * | | | | | | | |
|--|--------------------|--|--------------------|--------------------|--|--------------------|--------------------|
| CAG _(n) | Fragment Size (bp) | | CAG _(n) | Fragment Size (bp) | | CAG _(n) | Fragment Size (bp) |
| 1 | 72 | | 36 | 177 | | 75 | 294 |
| 2 | 75 | | 37 | 180 | | 80 | 309 |
| 3 | 78 | | 38 | 183 | | 85 | 324 |
| 4 | 81 | | 39 | 186 | | 90 | 339 |
| 5 | 84 | | 40 | 189 | | 95 | 354 |
| 6 | 87 | | 41 | 192 | | 100 | 369 |
| 7 | 90 | | 42 | 195 | | 105 | 384 |
| 8 | 93 | | 43 | 198 | | 110 | 399 |
| 9 | 96 | | 44 | 201 | | 115 | 414 |
| 10 | 99 | | 45 | 204 | | 120 | 429 |
| 11 | 102 | | 46 | 207 | | 125 | 444 |
| 12 | 105 | | 47 | 210 | | 130 | 459 |
| 13 | 108 | | 48 | 213 | | 135 | 474 |
| 14 | 111 | | 49 | 216 | | 140 | 489 |
| 15 | 114 | | 50 | 219 | | 145 | 504 |
| 16 | 117 | | 51 | 222 | | 150 | 519 |
| 17 | 120 | | 52 | 225 | | 155 | 534 |
| 18 | 123 | | 53 | 228 | | 160 | 549 |
| 19 | 126 | | 54 | 231 | | 165 | 564 |
| 20 | 129 | | 55 | 234 | | 170 | 579 |
| 21 | 132 | | 56 | 237 | | 175 | 594 |
| 22 | 135 | | 57 | 240 | | 180 | 609 |
| 23 | 138 | | 58 | 243 | | 185 | 624 |
| 24 | 141 | | 59 | 246 | | 190 | 639 |
| 25 | 144 | | 60 | 249 | | 195 | 654 |
| 26 | 147 | | 61 | 252 | | 200 | 669 |
| 27 | 150 | | 62 | 255 | | 205 | 684 |
| 28 | 153 | | 63 | 258 | | 210 | 699 |
| 29 | 156 | | 64 | 261 | | 215 | 714 |
| 30 | 159 | | 65 | 264 | | 220 | 729 |
| 31 | 162 | | 66 | 267 | | 225 | 744 |
| 32 | 165 | | 67 | 270 | | 230 | 759 |
| 33 | 168 | | 68 | 273 | | 235 | 774 |
| 34 | 171 | | 69 | 276 | | 240 | 789 |
| 35 | 174 | | 70 | 279 | | 245 | 804 |

*Size of PCR fragment in base pairs (bp) that can be expected when using the CAG primer mix A that has been provided. The formula for determining PCR fragment A size is $72 + 3n$, where n = the number of CAG repeats.

Material Supplied/Procedure

Important Information

Genotyping using this kit requires use of the appropriate fluorescent genetic analyzer instrument(s) and software capable of detection of fluorescently labeled fragments of varying lengths. This kit has been optimized for an ABI3100 genetic analyzer.

Procedure: SCA2 CAG Repeats Analysis by PCR

Thaw individual components. *Promptly store at -20°C after use.* Prepare **fresh** before use enough PCR premix for the number of reactions to be performed. Prepare 10% more for pipetting allowance. Prepare premix following the volumes given below. Follow the same ratio for preparing other final volumes.

PCR Thermal Cycler Files: Program the following PCR thermal cycler files

| Hot Start File | | |
|----------------|---------------------------|--------|
| Step | Time and Temperature | Cycles |
| Denaturation | 5 minutes at 98°C | 1 |
| Hold | Hold for infinity at 62°C | Hold |

| SCA2 Amplification File | | |
|--|--------------------------|-------------------|
| Step | Time and Temperature | Cycles |
| Initial Denaturation | 5 minutes at 95°C | 1 |
| Denaturation | 30 seconds at 94°C | 30 |
| Annealing | 30 seconds at 62°C | |
| Extension* | 60 seconds at 72°C | |
| Fill up | 7 minutes at 72°C | 1 |
| Hold | Hold for infinity at 4°C | Hold for infinity |
| *For CAG expansion greater than 70, the extension time can be increased to 1 minute. | | |

A. PCR premix preparation

Given below is a protocol for preparing a PCR premix for 25 µL reactions. This can be scaled up as required.

| PCR Premix Preparation | | |
|---|----------------|------------------|
| Component | 1 x 25 µL rxn. | 10 x 25 µLrxns.* |
| Sterile water | 8.0 µL | 80 µL |
| SCA2 Genemer™ Component A | 5.5 µL | 55 µL |
| PCR Component E | 7.5 µL | 75 µL |
| PCR Component M | 4.0 µL | 40 µL |
| Total | 25 µL | 250 µL |
| *Take into account that 3 µL/reaction will be used to prepare Taq polymerase mix (Enzyme Mix, EM) | | |

B. Enzyme premix (for 10 reactions). Label tube as “EM” (Enzyme mix)

| Enzyme Mix Preparation-EM | |
|---|----------------|
| Component | Volume |
| PCR premix (from above) | 30 µL |
| Taq. Polymerase* | 2.5 µL |
| Total | 32.5 µL |
| *The kit is optimized for regular Taq Polymerase. Any good quality Taq polymerase that is regularly used in the laboratory can be used. | |

C. Initial Denaturation/ Hot Start PCR

For each sample add the following

| Hot Start PCR | |
|--|--------|
| Component | Volume |
| PCR premix (above) | 21 µL |
| DNA Template* (~100ng chromosomal DNA) | 1 µL |
| * The SCA2 Genemer™ Control DNA [40-2038-01S] included with the kit is validated to amplify a fragment corresponding to 22 Repeats (19 CAG+2 CAA repeats). A volume of 1 µL can be used as a control reaction. | |

Transfer all sample tubes to thermal cycler and start “Hot Start” file. This step is used when using standard Taq polymerase. This step can be skipped if using hot start Taq polymerase.

D. PCR Amplification

After initial denaturation while thermal cycler is ‘holding’ at 62°C add Enzyme Mix-EM as follows to each tube.

| PCR Amplification | |
|---|--------|
| Component | Volume |
| Enzyme Premix-EM (from step B above) | 3 µL |

Start thermal cycling amplification file.

E. Post PCR Processing

Process sample for [Omni-Clean](#) purification of amplified products. This purification method is preferred over Sephadex G-50 spin column chromatography or ethanol precipitation as it is the most effective way to reduce the fluorescent primers and shorter primer dimers if present. [Omni-Clean Kit](#) is available from Gene Link, catalog number 40-4130-10 or 40-4140-10.

OR

Process sample for Sephadex G-50 spin column chromatography. G-50 columns should be equilibrated with sterile water.

OR

Process sample for ethanol precipitation.

Dissolve purified fragments in 20 µL water. Ready for fragment analysis.

F. Capillary Electrophoresis Fragment Analysis

Follow genetic analyzer manufacturer's instructions. Run two aliquots of sample at different concentrations. A brief protocol is given below for ABI3100 instrument.

1. Add 0.5 µL of appropriate size standards.
2. Add 1 & 2 µL of sample in duplicate tubes.
3. Add 12 µL of formamide loading buffer.
4. Mix and heat denature at 95°C for 5 minutes.
5. Transfer to ice.
6. Samples ready to load on instrument.

G. Gel Electrophoresis Analysis

PCR products can be analyzed by 2.5% agarose gel electrophoresis or by 10% polyacrylamide gel electrophoresis. An aliquot of 5-10 µL can be directly analyzed by adding non-denaturing loading (Gene Link product; Loading buffer 10X BPB/XC non-denaturing; 15 mL; Catalog #: 40-3003-15).

H. CAG Size Standards

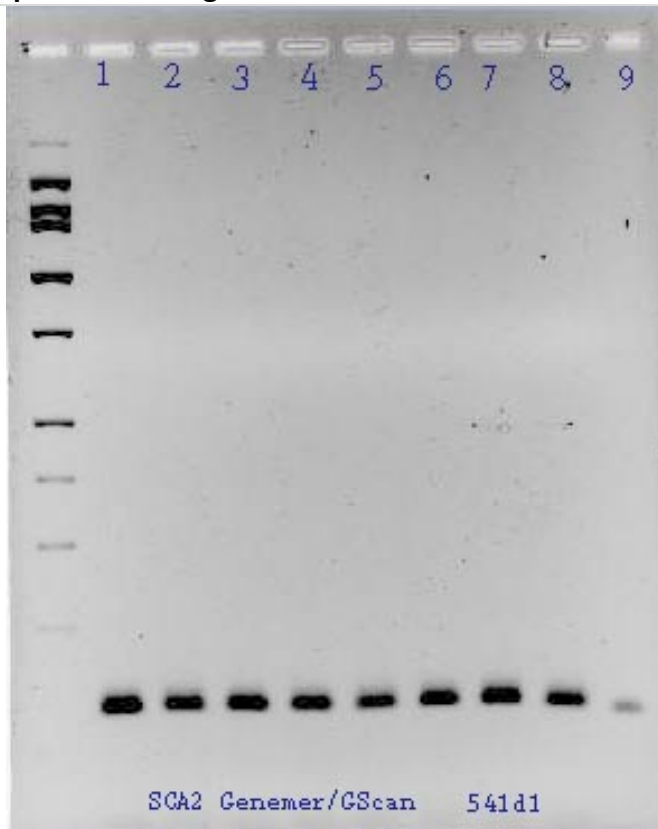
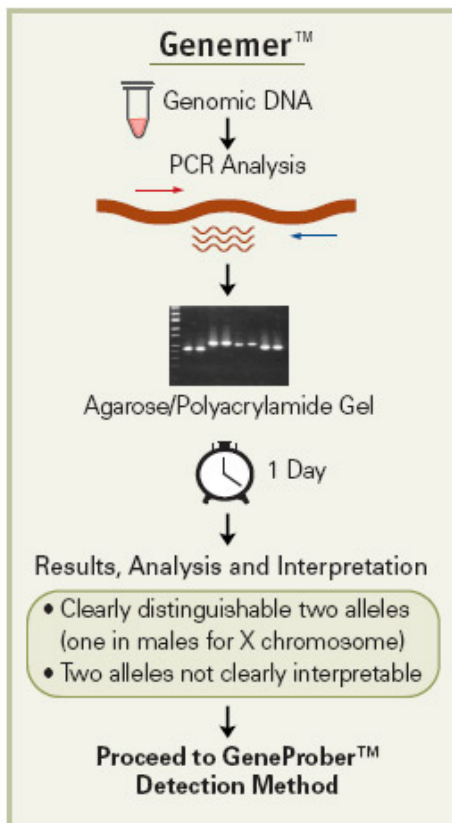
Due to their high GC content and the increased probability of secondary structure, fragments containing the CAG expansion tend to migrate faster during gel electrophoresis than corresponding molecular weight standards that usually have random GC contents. Furthermore, electrophoretic mobility increases with the size of the CAG expansion. A control SCA2 Genemer™ control DNA is included in this kit. Please refer to page 2 of this manual for exact specifications. We recommend that 1-2 µL of this control DNA used for amplification.

To correct for the anomalous migration rate of the CAG fragments, Gene Link provides control DNA samples and GScan™ ready to run samples of various CAG sizes. Please refer to product information page for details. The CAG size standards were generated by PCR using the primers provided with this kit and template DNA of the *ATXN2* gene that contained CAG repeats of known size. The number of CAG repeats was verified by sequence analysis. The stability of size repeats upon cloning and amplification has NOT been determined. Thus, the size should be considered approximate and there is no claim for each fragment to contain the exact number of triple repeats. These control DNA's are sold with the express condition that these NOT be used for exact triple repeat size determination of DNA of unknown genotype. The control DNA should be used for determining the performance of specific GScan™, Genemer™ and PCRProber™ Gene Link products.

SCA2 Genotyping Results and Interpretation

| SCA Type | Normal Repeats | Intermediate Length | Full Disease Length |
|---|----------------|---------------------|---------------------|
| Spinocerebellar ataxia 1 (SCA1) | 6-39 | None Reported | 40-81 |
| Spinocerebellar ataxia 2 (SCA2) | 14-31 | None Reported | 34-59 |
| Spinocerebellar ataxia 3 (SCA3)/ Machado Joseph disease (MJD) | 13-40 | None Reported | 60-84 |
| Spinocerebellar ataxia 6 (SCA6) | 4-20 | None Reported | 21-28 |
| Spinocerebellar ataxia 7 (SCA7) | 7-19 | 28-35 | 38-220 |

SCA2 GScan™ [40-2038-15FM] & Genemer™ V2 Kit [40-2038-11] PCR Amplification & Agarose Gel Detection



SCA2 X GScan™ V2 [40-2038-15FM] and SCA2 Genemer™ V2 Kit [40-2038-11] was used to amplify normal human genomic samples (lanes 1-8) and the SCA2 Genemer control template DNA (lane 9) containing 19 CAG and 3 CAA repeat. Approximately 130 bp fragment was amplified from the control DNA template and all normal fragment sizes from the 8 human genomic samples. A 25 µL volume PCR amplification was performed using SCA2 GScan™ kit. After PCR 10 µL samples were applied to a 2.5 agarose gel for electrophoresis. Gel picture is of an ethidium bromide stained gel.

Appendix: Protocols

Genomic DNA Purification

Genomic DNA is usually extracted from blood. A simple procedure is given below that purifies ~10 µg DNA from 300 µl blood using a 30 minute procedure.

Omni-Pure™ Genomic DNA Purification System Catalog Number: 40-4010-01

A. Initial Preparation

1. Label two sets of 1.5 ml tubes per sample.
2. Add 900 µl GD-1 solution (RBC Lysis Solution) to one tube for each sample.
3. Add 300 µl Isopropanol (2-propanol) to one tube for each sample. Cap the tubes.

B. Cell Lysis

1. To the tube containing 900 µl GD-1 solution (RBC Lysis Solution) using a filter tip pipet transfer 300 µl whole blood. Cap and gently mix by inversion. Incubate for 1-3 minutes at room temperature. Mix by inversion a few times during this incubation period. Incubate longer for fresh blood cells as they are intact and not lysed already.
2. Centrifuge at 3 K rpm for 20 seconds to pellet the white blood cells. A reddish white pellet should be clearly visible. Decant and discard supernatant leaving behind the last few droplets. Do not totally remove the supernatant.
3. Completely resuspend the white blood cell pellet by vigorously vortexing the tube. Ensure that the pellet is completely resuspended.
4. To the resuspended cells add 300 µl GD-2 solution (Cell Lysis Solution). Mix by gentle vortexing. You will notice release of DNA by the thickening of the liquid in the sample. Samples may be stored at this stage for processing later. It has been shown that the samples are stable in Cell Lysis Solution for at least 2 years at room temperature.

C. Protein Precipitation

1. Add 100 µl GD-3 solution (Protein Precipitation Solution) to the sample in cell lysis solution.
2. Vortex vigorously for 20 seconds. Small particles of brown color will appear and be visible at this stage.
3. Centrifuge at 5 K rpm for 1 minute to pellet the precipitated proteins. A clearly visible brown pellet containing proteins should be collected at the bottom of the tube.

D. DNA Precipitation

1. Decant the supernatant containing the DNA to a new appropriately labeled tube (see initial preparation above) containing 300 µl 100% Isopropanol (2-propanol).
2. Mix the sample by inversion until a visible white floating DNA strand-particle is identified. Mixing by inversion 30-40 is usually sufficient.
3. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. A white DNA pellet should be clearly visible.
4. Decant supernatant and place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining supernatant.
5. To remove residual salts, add 300 µl of 70% ethanol. Vortex gently.
6. Centrifuge at 6 K rpm for 1 minute to collect the DNA as a pellet. Gently take out the tubes so that the pellet is not dislodged. While holding the tube, rotate tube so that you can watch the pellet. Now carefully decant the ethanol, keeping an eye on the pellet so that it does not flow away.
7. Place tube inverted on a clean Kimwipe™ tissue paper to drain the remaining ethanol.
8. Air dry the DNA pellet. Do not use vacuum.

E. DNA Reconstitution & Use

1. Add 100 µl of GD-4 solution (DNA Reconstitution Solution). Vortex gently. Incubate at 60°C for 5 minutes to facilitate dissolution or keep overnight at room temperature.
2. Store DNA at 4 °C. For long-term storage, place sample at -20 °C or -80 °C.
3. Average yield of 10 µg is expected from 300 µl blood DNA. The range is between 5 µg to 15 µg.
4. The 100 µl of purified DNA obtained will have an average concentration of ~ 100 ng/µl.
5. For PCR amplification use 1-2 µl.
6. Use 100 µl for restriction digestion followed by Southern blot analysis.
7. It is convenient to perform multiple 300 µl blood DNA purification instead of scaling up the procedure.

SCA2 Genotyping Product Ordering Information

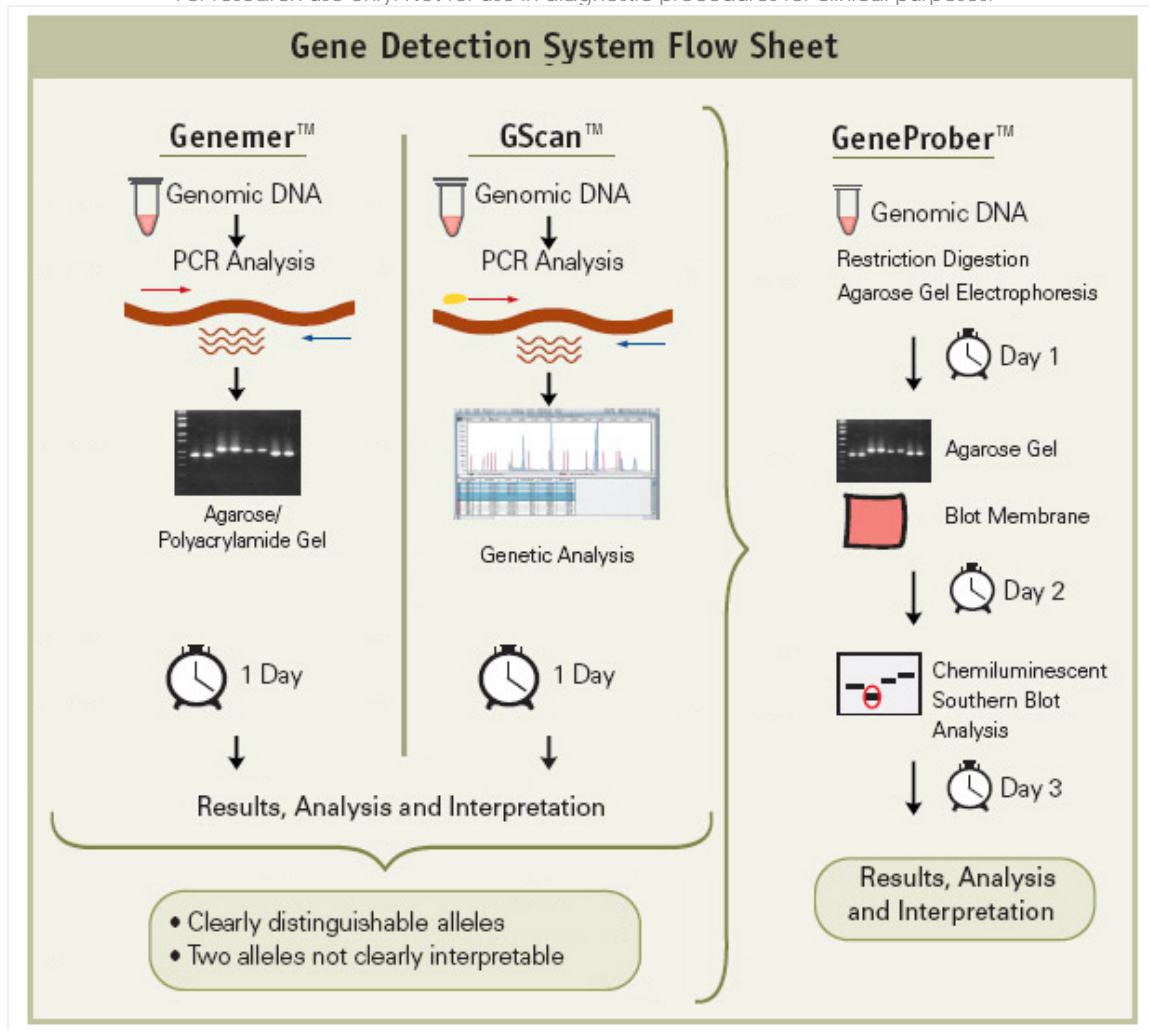
| Product | Unit Size | Catalog No. |
|---|---------------------|--------------|
| SCA2 Genemer™ Kit for gel based detection. Kit for performing PCR amplification and gel based detection. | 1 Kit [100 rxns] | 40-2038-11 |
| SCA2 GScan™ Kits for Fam fluorescent detection Kit for performing fluorescent PCR amplification based detection. | 1 Kit [100 rxns] | 40-2038-15FM |
| SCA2 Genemer™ Primer pair Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions. | 10 nmols | 40-2038-10 |
| SCA2 22 repeat (19 CAG + 3 CAA) GScan™ & Genemer™ Control DNA | 500 ng | 40-2038-01 |

Fragile X Genotyping Product Ordering Information

| Product | Unit Size | Catalog No. |
|---|----------------------|--------------|
| Fragile X Genemer™ Kit for gel based detection. Kit for performing PCR amplification and gel based detection. | 1 Kit [100 rxns] | 40-2004-11 |
| Fragile X GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection. Various dye kits. XX=FM for 6-Fam; HX for Hex; TT for Tet; C3 for Cy3 and C5 for Cy5. | 1 Kit [100 rxns] | 40-2004-15XX |
| Fragile X GeneProber™ GLFX1 Probe unlabeled Probe for radioactive labelling and Southern blot analysis | 500 ng | 40-2004-40 |
| Fragile X GeneProber™ GLFX1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis | 110 µL | 40-2004-41 |
| Fragile X Genemer™ Primer pair Primers for amplification of CGG triple repeat spanning region. The quantity supplied is sufficient for 400 regular 50 µL PCR reactions. | 10 nmols | 40-2004-10 |
| Fragile X PCRProber™ AP labeled probe Alkaline phosphatase labeled probe | 12 µL | 40-2004-31 |
| Fragile X PCRProber™ Kit for chemiluminescent detection Kit for performing PCR amplification and chemiluminescent based detection. | 5 blots [50 rxns] | 40-2004-32 |

FRAXE/FMR2/AFF2 Genotyping Product Ordering Information

| Product | Unit Size | Catalog No. |
|---|---------------------|--------------|
| FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1 Probe Digoxigenin labeled Probe for non-radioactive chemiluminescent Southern blot analysis | 110 µL | 40-2054-41 |
| FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection Kit for performing PCR amplification & gel based detection | 1 Kit [100 rxns] | 40-2054-11 |
| FRAXE/FMR2/AFF2 GScan™ Kits for fluorescent detection Kit for performing fluorescent PCR amplification based detection, Fam labeled | 1 Kit [100 rxns] | 40-2054-15FM |
| Fragile X ~16 CGG repeat Genemer Control Template DNA | 500 ng | 40-2004-01 |
| Fragile X ~29 CGG repeat Genemer Control Template DNA | 500 ng | 40-2004-02 |
| Fragile X ~40 CGG repeat Genemer Control Template DNA | 500 ng | 40-2004-03 |
| Fragile X ~60 CGG repeat Genemer Control Template DNA | 500 ng | 40-2004-04 |
| Fragile X ~90 CGG repeat Genemer Control Template DNA | 500 ng | 40-2004-05 |



Genemer™ Kits Product Ordering Information

Gene Link's Genemer™ kits contain optimized PCR amplification components for convenient agarose or polyacrylamide genotyping of triple repeat disorders and other genetic disorders. These are safe, convenient and sensitive, and afford rapid screening of samples. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. Included in these kits are ready-to-run control samples of various repeats of the specific triple repeat disorder.

The Genemer™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

| Product | Unit Size | Catalog No. |
|---|-----------|-------------|
| Fragile X Genemer™ V2 Kit for gel based detection; 100 reactions kit | 1 kit | 40-2004-11 |
| FRAXE/FMR2/AFF2 Genemer™ Kit for gel based detection; 100 reactions kit | 1 kit | 40-2054-11 |
| Huntington's Disease Genemer™ V2 Kit for gel based detection; 100 reactions kit kit | 1 kit | 40-2025-11 |
| Myotonic Dystrophy Genemer™ Kit for for gel based detection; 100 reactions kit | 1 kit | 40-2026-11 |
| Friedreich's Ataxia Genemer™ Kit for gel based detection; 100 reactions kit | 1 kit | 40-2027-11 |

All Gene Link products are for research use only

Current pricing are posted at <http://www.genelink.com/>

GeneProber™ Related Product Ordering Information

The GeneProber™ product line is based on the chemiluminescent Southern blot detection method. Gene Link's non-radioactive detection systems for genotyping of triple repeat disorders are rapid, reliable and as sensitive as the ³²P labeled southern blots. No more decayed probes and radioactive exposure. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders.

Unlabeled GeneProber™ probes are also available for radio labeling and radioactive based detection. Gene Link strongly recommends the use of non-radioactive gene detection systems. Consider switching to Gene Link's product line of non-radioactive detection systems.

| Product | Unit Size | Catalog No. |
|---|-----------|-------------|
| Fragile X GeneProber™ GLFX1 Probe unlabeled | 500 ng | 40-2004-40 |
| Fragile X GeneProber™ GLFXDig1 Probe Digoxigenin labeled | 110 µL | 40-2004-41 |
| FRAXE/FMR2/AFF2 GeneProber™ AFF2-AJ31Dig1 | 110 µL | 40-2054-41 |
| Huntington's Disease GeneProber™ GLHD14 Probe unlabeled | 500 ng | 40-2025-40 |
| Huntington's Disease GeneProber™ GLHDDig2X Probe Digoxigenin labeled | 110 µL | 40-2025-41 |
| Myotonic Dystrophy GeneProber™ GLDM1 Probe unlabeled | 500 ng | 40-2026-40 |
| Myotonic Dystrophy GeneProber™ GLDMDig2 Probe Digoxigenin labeled | 110 µL | 40-2026-41 |
| Friedreich's Ataxia GeneProber™ GLFRDA21 Probe unlabeled | 500 ng | 40-2027-40 |
| Friedreich's Ataxia GeneProber™ GLFRDADig21 Probe Digoxigenin labeled | 110 µL | 40-2027-41 |

GScan™ Kits Product Ordering Information

Gene Link's GScan™ gene detection products are safe, convenient and sensitive, and afford automated compilation of data. Kits are available for reliable genotyping of the Fragile X, Huntington's Disease, Myotonic dystrophy and other triple repeat mutation group disorders. The kits contain optimized PCR amplification reagents and a wide array of fluorescent-labeled primers for genotyping after PCR using fluorescent genetic analyzer instrument. Included in these kits are ready-to-run control samples of various repeats of the triple repeat disorder kit. These control samples are for calibration with the molecular weight markers for accurate size determination of the amplified fragments.

The GScan™ kits are simple and robust for routine triple-repeat detection of greater than 100 repeats of all triple repeat disorders listed.

| Product | Unit Size | Catalog No. |
|---|-----------|---------------|
| Fragile X GScan™ V2 Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2004-15XX |
| Fragile X GScan™ V2 Kit for fluorescent detection; 20 reactions kit | 1 kit | 40-2004-15FMS |
| FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2054-15FM |
| FRAXE/FMR2/AFF2 GScan™ Kit for fluorescent detection; 20 reactions kit | 1 kit | 40-2054-15FMS |
| Huntington's Disease GScan™ V2 Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2025-15XX |
| Huntington's Disease GScan™ V2 Kit for fluorescent detection; 20 reactions kit | 1 kit | 40-2025-15FMS |
| Myotonic Dystrophy GScan™ Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2026-15XX |
| Myotonic Dystrophy GScan™ Kit for fluorescent detection; 20 reactions kit | 1 kit | 40-2026-15FMS |
| Friedreich's Ataxia GScan™ Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2027-15FM |
| Friedreich's Ataxia GScan™ Kit for fluorescent detection; 20 reactions kit | 1 kit | 40-2027-15FMS |
| SCA2 GScan™ Kit for fluorescent detection; 100 reactions kit | 1 kit | 40-2038-15FM |

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Southern Blot Buffers & Reagents

| Product | Catalog No. | Unit Size |
|--|-------------|-------------|
| Agarose Tablets, 0.5 gm each; 100 tablets | 40-3011-10 | 100 tablets |
| Agarose LE Molecular Biology Grade; 100 g | 40-3010-10 | 100 g |
| Agarose LE Molecular Biology Grade; 500 g | 40-3010-50 | 500 g |
| Hybwash A, Hybridization Wash Solution (20X SSC); 200 mL | 40-5020-20 | 200 mL |
| Hybwash B, Hybridization Wash Solution (10% SDS); 100 mL | 40-5021-10 | 100 mL |
| TAE Buffer; 50 X Concentrate; 100 mL | 40-3007-01 | 100 mL |
| TAE Buffer; 50 X Concentrate; 1 L | 40-3007-10 | 1 L |
| TBE Buffer; 5 X Concentrate; 1 L | 40-3008-10 | 1 L |
| Buffer M 10X (Maleic Acid buffer); 100 mL | 40-5025-10 | 100 mL |
| 10% Blocking solution; 100 mL | 40-5026-10 | 100 mL |
| Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL | 40-5027-10 | 1 mL |
| 10x AP Detection buffer (alkaline phosphatase detection buffer); 100 mL | 40-5031-10 | 100 mL |
| Lumisol™ I Hybridization Solution; contains formamide; 200 mL | 40-5022-20 | 200 mL |
| Lumisol™ II Hybridization Solution; for non-toxic hybridizations; 200 mL | 40-5023-20 | 200 mL |
| Lumisol™ III Hybridization Solution; for oligo probes; 200 mL | 40-5024-20 | 200 mL |
| CDP-Star® Substrate; Ready-to-Use 0.25 mM in spray bottle; 10 mL | 40-5010-10 | 10 mL |

Loading Buffers

| Product | Catalog No. | Size |
|---|-------------|-------|
| Gel Loading Buffer 5X BPB/XC non-denaturing; 1 mL | 40-3002-10 | 1 mL |
| Gel Loading Buffer 5X BPB/XC non-denaturing; 15 mL | 40-3002-15 | 15 mL |
| Gel Loading Buffer 10X BPB/XC non-denaturing; 1 mL | 40-3003-10 | 1 mL |
| Gel Loading Buffer 10X BPB/XC non-denaturing; 15 mL | 40-3003-15 | 15 mL |
| Gel Loading Buffer 5X Orange G/XC non-denaturing; 1 mL | 40-3004-10 | 1 mL |
| Gel Loading Buffer 5X Orange G/XC non-denaturing; 15 mL | 40-3004-15 | 15 mL |
| Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 1 mL | 40-5027-10 | 1 mL |
| Gel Loading Buffer 2X BPB/XC Denaturing for Sequencing; 15 mL | 40-5027-15 | 15 mL |
| DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer ; 1 mL | 40-5028-10 | 1 mL |
| DNA SDS Gel Loading Buffer 5X BPB/XC DNA binding protein denaturing buffer; 15 mL | 40-5028-15 | 15 mL |
| RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 1 mL | 40-5029-10 | 1 mL |
| RNA Gel Loading Buffer 2X BPB/XC with ethidium bromide; 15 mL | 40-5029-15 | 15 mL |
| RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide ; 1 mL | 40-5030-10 | 1 mL |
| RNA Gel Loading Buffer 2X BPB/XC without ethidium bromide; 15 mL | 40-5030-15 | 15 mL |

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