KASP genotyping technology

For Research Use Only. Not for use in diagnostic procedures.
An introduction to KASP

KASP™ genotyping technology from LGC, Biosearch Technologies™ utilises a unique form of competitive allele-specific PCR (polymerase chain reaction) that enables highly accurate bi-allelic scoring of SNPs (single nucleotide polymorphisms) and indels (insertions/deletions) at specific loci across a wide range of genomic DNA samples, including those of complex genomes. KASP technology delivers extremely high levels of assay robustness and accuracy with significant cost savings.

**Superb accuracy and performance**

- Accuracy >99.8% based on independent assessment
- Industry leading SNP and InDel assay conversion rate (>90%).

**Tremendous flexibility**

- Flexible primer design which increases the rate of successful assay development
- Supports low-, medium- and high-throughput studies and individual repeat assays
- Compatible with a wide range of liquid handling systems and thermal cyclers; signal can be read on most FRET (fluorescent resonant energy transfer) capable plate readers and qPCR machines.

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**How KASP works**

1. **Assay components:**
   - **A) KASP Assay Mix:** consists of 2 allele specific primers and 1 reverse primer.
   - **B) KASP-TF Master Mix:** contains universal fluorescent probes, Taq polymerase and dNTP’s in an optimised buffer solution.
   - **C) Sample DNA:** DNA contains the SNP of interest.

   ![](https://via.placeholder.com/150)

   **Legend**
   - Allele-1 tail FAM-labelled oligo sequence
   - Allele-2 tail HEX-labelled oligo sequence
   - Common reverse primer
   - FAM dye
   - HEX dye
   - Target SNP
   - Quencher

2. **Denatured template and annealing components – PCR round 1:**

   (Reverse primer primers, elongates and makes a complementary copy of the allele-1 tail.)

3. **Complement of allele-specific tail sequence generated – PCR round 2:**

   Assembled as a homogeneous assay in 96-, 384- or 1536-well PCR plates, bi-allelic discrimination is achieved through the competitive binding of two allele-specific forward primers, each with a unique tail sequence that corresponds with one of two universal probes; one labelled with FAM™ dye and the other with HEX™ dye.

4. **Signal generation – PCR round 3:**

   In further rounds of PCR, levels of allele-specific tail increase. The fluor labelled part of the FRET cassette is complementary to new tail sequences and binds, releasing the fluor from the quencher to generate a fluorescent signal.

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**Breakthrough simplicity and flexibility**

- KASP uses a universal reporting system, which simplifies assay design and eliminates time-intensive probe design steps
- Requires only 10 ng DNA per sample per SNP (based on human genome size)
- Low reaction volumes keep reagent costs to a minimum
- Cost benefits enable you to perform more assays overall, improving the quality of your data.

KASP genotyping chemistry is available as a service in Europe and North America or as in silico wet lab validated and non-validated kits.

<table>
<thead>
<tr>
<th>KASP by Design (KBD)</th>
<th>KASP on Demand (KOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Designed in silico</td>
<td>Designed in silico</td>
</tr>
<tr>
<td>No validation</td>
<td>Optimised and validated</td>
</tr>
</tbody>
</table>

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1. KASP Assay Mix

The KASP Assay Mix contains three assay-specific non-labelled primers: two allele-specific forward primers plus one common reverse primer. The sequences for these primers are generated via our proprietary Kraken™ software. KASP Assay Mix can be purchased in two formats:

1. **KASP by Design (KBD)** – designed in silico
2. **KASP on Demand (KOD)** – validated and optimised in our laboratories. KOD assays are guaranteed to generate accurate genotyping data when used in conjunction with DNA of suitable quality and KASP-TF Master Mix.

KASP Assay Mix is supplied in a single 2D barcoded tube containing sufficient Assay mix to run a minimum of 2500 x 10 µL reactions - the recommended volume for 96-well PCR plates. If run in 384-well PCR plates, the total reaction volume can be reduced to 5 µL, doubling the number of reactions and cutting the cost per data point in half.

For breakthrough savings, customers can utilise our SNPline workflow for high-throughput PCR, that we developed for use in our own service laboratories and that enables 1 µL reactions in 1536-well PCR plates. SNPline workflow customers can achieve a 10-fold reduction in the cost per data point, while increasing throughput up to 500,000 data points per day.

2. KASP-TF Master Mix

KASP-TF Master Mix is formulated without Triton™ X-100 (Triton-Free), and contains the universal FRET cassettes, ROX™ passive reference dye, KASP™Taq™ DNA polymerase, free nucleotides and MgCl₂ in an optimised buffer solution. KASP-TF Master Mix is universal to every KASP genotyping assay and is offered in a range of standard packaging sizes to address varying throughput needs. Custom packaging solutions are also available if needed.

### KASP genotyping components

**KASP genotyping assay components**

Designing a KASP genotyping assay is fast and easy. KASP uses a universal reporting system that eliminates the need to design assay-specific fluorescent probes. Simply create your Assay mix consisting of your assay-specific primers, add your DNA sample and KASP-TF Master Mix. You can run KASP in 96-, 384- or 1536-well PCR plates. To run, seal the plate and run your PCR cycling protocol. At the end of the thermal cycle, conduct an end-point PCR read using either a qPCR instrument or FRET-capable plate reader. No separation by agarose gel is required.

### 2. KASP-TF Master Mix

KASP-TF Master Mix is universal to every KASP genotyping assay and is offered in a range of standard packaging sizes to address varying throughput needs. Custom packaging solutions are also available if needed.

### KASP genotyping workflow

**Assembly of KASP genotyping reactions in 96-, 384- or 1536-well PCR plates**

- **DNA samples**
  - DNA extracted from plant, animal, human or microbial species
  - 10 ng of DNA required per reaction (based on human genome size)

- **KASP Assay Mix**
  - Three target specific primers per assay (no labelling required)
  - Two allele-specific forward primers
  - One reverse primer
  - Each SNP or InDel assay provided in a single 2D barcoded tube

- **KASP-TF Master Mix**
  - FRET reporting system (universal FAM and HEX labelled cassettes)
  - DNA polymerase
  - ROX passive reference dye
  - dNTPs
  - MgCl₂
  - Optimised buffer

Following completion of the KASP PCR, reaction plates are read and the data analysed using any cluster analysis viewing software. Detected signals are plotted as a graph, with samples of the same genotype clustering together. In our genotyping service laboratories, cluster analysis is performed using our proprietary Kraken software. An example of the data generated is shown on the right.

- **Heat/laser/pressure sealing of plates**
- **Fluorescent reading of plates**
- **PCR thermal cycle**
- **Cluster analysis - assign genotypes to samples**
- **Data export**

**Cluster analysis - assign genotypes to samples**

- **Red** Sample is homozygous for the HEX allele
- **Green** Sample is heterozygous: one FAM allele and one HEX allele
- **Blue** Sample is homozygous for the FAM allele

**KASP genotyping reaction**

- **KASP Assay Mix**
- **KASP-TF Master Mix**
- **Sample DNA**
**KASP products and services**

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### Our proprietary Kraken software

The Kraken software has an industry-leading SNP-to-assay conversion rate that exceeds 90%. This means that, on average, 9 out of 10 KBD assays will work based on standard conditions, without any optimisation.

### Once validation has been completed

If validation has been completed for a particular SNP, it does not need to be repeated. When a customer orders a set of assays as KOD products, the next time they order this same set, they would order the assays as KBD products since the validation work has already been completed. The same holds true for service orders; the set-up charge applies only to the first order.

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### Product options

<table>
<thead>
<tr>
<th>Service</th>
<th>KBD - KASP by Design</th>
<th>KOD - KASP on Demand</th>
<th>Genotyping service using KASP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative cost and value provided</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Lead times</td>
<td>1-3 weeks</td>
<td>Standard: 4-6 weeks</td>
<td>Quoted based on scope</td>
</tr>
<tr>
<td></td>
<td>Fast: 1-3 weeks</td>
<td></td>
<td>(of SNP/seq of samples)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Typically 4-6 weeks</td>
</tr>
<tr>
<td>Customer submits reference sequences</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Target-specific KASP primer sequences are designed for each assay using proprietary Kraken software</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Target specific primer sequences and common reverse primers prepared</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Primers pooled into a single 2D barcoded tube to create the KASP Assay Mix</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Assay validation is performed in our service lab with customer-supplied DNA. Should any assay fail, assay conditions and/or primer sequences will be optimised until the assay works</td>
<td>No1</td>
<td>Yes2</td>
<td>Yes2</td>
</tr>
<tr>
<td>Biosearch Technologies provides a money-back guarantee that each assay will work</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Biosearch Technologies service lab runs customer samples and provides genotyping data</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

1. "Our proprietary Kraken software has an industry-leading SNP-to-assay conversion rate that exceeds 90%. This means that, on average, 9 out of 10 KBD assays will work based on standard conditions, without any optimisation.
2. Once validation has been completed for a particular SNP, it does not need to be repeated. If a customer orders a set of assays as KOD products, the next time they order this same set, they would order the assays as KBD products since the validation work has already been completed. The same holds true for service orders; the set-up charge applies only to the first order.

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### Ordering information

**KASP Assay Mix**

<table>
<thead>
<tr>
<th>Catalogue number</th>
<th>Description and quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBS-2300-001</td>
<td>KASP by Design Primer Mix, 2500 x 10 μL Reactions, Standard Service</td>
</tr>
<tr>
<td>KBS-2300-002</td>
<td>KASP by Design Primer Mix, 2500 x 10 μL Reactions, Standard Service, Manual Design</td>
</tr>
<tr>
<td>KBS-2400-001</td>
<td>KASP on Demand Primer Mix, 2500 x 10 μL Reactions, Standard Service</td>
</tr>
<tr>
<td>KBS-2400-002</td>
<td>KASP on Demand Primer Mix, 2500 x 10 μL Reactions, Rapid Service</td>
</tr>
<tr>
<td>KBS-2400-003</td>
<td>KASP on Demand Primer Mix, 2500 x 10 μL Reactions, Standard Service Manual Design (3 Primers)</td>
</tr>
</tbody>
</table>

**KASP-TF Master Mix examples**

<table>
<thead>
<tr>
<th>Catalogue number</th>
<th>Description and quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBS-1050-101</td>
<td>KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (2.5 mL)</td>
</tr>
<tr>
<td>KBS-1050-102</td>
<td>KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (25 mL)</td>
</tr>
<tr>
<td>KBS-1050-103</td>
<td>KASP-TF V4.0 2X Master Mix 96/384, Standard ROX (250 mL) (10 x 25 mL)</td>
</tr>
<tr>
<td>KBS-1050-111</td>
<td>KASP-TF V4.0 2X Master Mix 1536, Standard ROX (2.5 mL)</td>
</tr>
<tr>
<td>KBS-1050-112</td>
<td>KASP-TF V4.0 2X Master Mix 1536, Standard ROX (25 mL)</td>
</tr>
<tr>
<td>KBS-1050-113</td>
<td>KASP-TF V4.0 2X Master Mix 1536, Standard ROX (250 mL) (10 x 25 mL)</td>
</tr>
<tr>
<td>KBS-1050-164</td>
<td>KASP-TF V4.0 Trial Kit (Standard ROX)</td>
</tr>
</tbody>
</table>

ROX is added to KASP-TF Master Mix as a passive reference dye. Different qPCR instruments have differing requirements for ROX; please contact techsupport@lgcgroup.com to determine the correct version for your instrument. Formulations for use with 1536-well PCR plates are compatible with SNPline™ instrumentation. Please enquire for ordering information.

**Software and barcode reading**

<table>
<thead>
<tr>
<th>Catalogue number</th>
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</tr>
</thead>
<tbody>
<tr>
<td>KBS-0101-002</td>
<td>KlusterCaller, genotyping calling software</td>
</tr>
<tr>
<td>KBS-0101-001</td>
<td>Kraken full LIMS system for genotyping (site license)</td>
</tr>
<tr>
<td>KBS-0025-001</td>
<td>Cyclops 2D single tube reader</td>
</tr>
<tr>
<td>KBS-0025-002</td>
<td>ZTS-01 96-rack 2D reader</td>
</tr>
</tbody>
</table>

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