

KASP reagents guidance notes



LGC

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

KASP reagents

We are pleased to provide guidance on ordering and storage of your KASP[™] genotyping reagents.

The following topics are covered in this document: **Section 1: KASP genotyping reagents**

- KASP Assay Mix
- KASP-TF Master Mix

Section 2: How to order KASP reagents Note A: Submitting DNA for KOD assay validation

Section 3: Shipment and storage of KASP reagents Section 4: Document links Section 5: Contact information Section 6: Delivery and payment information

Section 1: KASP genotyping reagents

KASP Assay Mix

KASP Assay Mix typically consists of three KASP primers, custom-designed to target the SNP or InDel of interest. Assay design is performed using our proprietary Kraken[™] software. LGC, Biosearch Technologies[™] offers three different formats of KASP Assay Mix that are detailed in Table 1. Each standard KBD or KOD Assay Mix provides enough material for 2,500 × 10 µL reactions.

Product code	Product name	Description
KBS-2300-001	KASP by Design (KBD)	Assay is designed, <i>in silico</i> -validated, and manufactured by Biosearch Technologies (not laboratory-validated). 2-3 week turnaround time.
KBS-2400-001	KASP on Demand (KOD) Standard service	Assay is designed, manufactured and laboratory-validated* by Biosearch Technologies. 4-6 weeks turnaround time.
KBS-2400-002	KASP on Demand (KOD) Rapid service	Assay is designed, manufactured and laboratory-validated* by Biosearch Technologies. 2-3 weeks turnaround time.

 Table 1. KASP Assay Mix formats available from Biosearch Technologies.

*Laboratory validation is performed using DNA samples submitted by the customer for all species except human. Human KASP Assays are validated using our in-house panel of 44 DNA samples.

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KASP-TF Master Mix

KASP-TF Master Mix contains the universal FRET cassettes, ROX[™] passive reference dye, *Taq* polymerase, free nucleotides and MgCl₂ in an optimised buffer solution. KASP-TF Master Mix is universal and can be used in conjunction with all KASP assays. Biosearch Technologies' KASP-TF Master Mix options are detailed in Table 2.

Product name	Description	Volumes available	ROX levels available*
KASP-TF V4.0 2X Master Mix	Sold with KBD/KOD assays. Suitable for use with most benchtop qPCR instruments and FRET-capable plate readers**	2.5 mL, 25 mL, 250 mL	Standard, High, Low

 Table 2. KASP-TF Master Mix formats available from Biosearch Technologies.

*To determine the appropriate ROX level for your instrument, please visit our website.

**Please note: if you are using a high-throughput platform (e.g. Nexar, SNPline or Fluidigm), please discuss the appropriate formulation of KASP-TF Master Mix with Biosearch Technologies prior to ordering.

To help calculate the volume of KASP-TF Master Mix needed for your experiments, below are the reagent volumes required for KASP genotyping mix for both 96-well and 384-well plates. Note that if the DNA samples are dried down, 2X KASP-TF Master Mix must be diluted with water to adjust the concentration to 1X.

Please see further details in our KASP Quick Start Guide and User Manual and in Section 5.

	Wet DNA method		Dry DNA method	
	98-well plate (μL per well)	384-well plate (μL per well)	96-well plate (µL per well)	384-well plate (μL per well)
DNA	5	2.5	n/a	n/a
2x KASP-TF Master Mix	5	2.5	5	2.5
KASP Assay Mix	0.14	0.07	0.14	0.07
Water	n/a	n/a	5	2.5
Total reaction volume	10	5	10	5

Table 3. Reagent volumes for preparing KASP genotyping mix.

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Section 2: How to order KASP reagents



* Email quote requests and completed forms to Customer Service for your region: Americas: <u>Genomics.Americas@lgcgroup.com</u> EMEA (Europe, Middle East and Africa): <u>Genomics.EMEA@lgcgroup.com</u> APAC (Asia, Pacific): <u>Genomics.APAC@lgcgroup.com</u>

** DNA submission form and sample DNA shipment required for non-human KOD Assays only. Human KOD projects utilise Biosearch Technologies' in-house human DNA panel, see <u>Note A</u>.

For ordering assistance, please contact our Customer Services team.

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Note A: Submitting DNA for KOD assay validation

For laboratory validation of human KOD assays, Biosearch Technologies will use an in-house panel of 44 human DNA samples. You are therefore not required to submit DNA samples for human KOD assays. If you are working on a low frequency polymorphism, we require control samples that are representative of each possible genotype to be submitted to enable results to be confirmed.

For laboratory validation of KOD assays for any species other than human, Biosearch Technologies requires a set of DNA samples from the customer. For guidelines on the amounts of DNA required, and how to prepare this, please view our <u>DNA requirements document</u> (see <u>Section 4</u>).

When submitting DNA, please provide expected genotypes for your samples for each assay. This can be provided in a list or table format (e.g. using MS Excel). Figure 1 gives an example of how to provide this information. If the genotypes of your validation samples are unknown, please discuss with your project manager prior to submitting your samples.

	KOD assay 1	KOD assay 2	KOD assay 3
Sample 1	A:A	C:C	G:T
Sample 2	A:A	C:C	G:T
Sample 3	A:A	C:C	G:G
Sample 4	A:A	C:C	T:T
Sample 5	A:G	C:C	G:G
Sample 6	G:G	C:C	G:T
Sample 7	G:G	C:C	T:T
Sample 8	A:A	C:T	T:T
Sample 9	A:A	C:T	G:G
Sample 10	A:G	C:T	G:T
Sample 11	A:A	C:T	Unknown
Sample 12	A:A	T:T	T:T

Figure 1. An example of how to provide expected genotype information to Biosearch Technologies for KOD assay validation.

Please note:

- For validation of KOD assays, a minimum of 22 unique DNA samples is required. The maximum number of DNA samples is 92, allowing four wells to be run as no template controls (NTCs).
- If DNA is not of sufficient quality for assay validation, and Biosearch Technologies have to repeat validation using replacement samples, this work will be charged at cost per data point.
- Assays that do not meet KOD validation criteria can be shipped as KBD assays at the customer's request.
- After completion of a KOD validation project, any remaining DNA will be destroyed after 3 months unless otherwise requested.

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Section 3: Shipment and storage of KASP reagents

KASP Assay Mix

KASP Assay Mix is shipped at ambient temperature. As KASP Assay Mix consists of unlabelled oligonucleotides, these will be stable at room temperature for a number of days if shipments are delayed for any reason. Upon receipt, KASP Assay Mix should be frozen (-20 °C).

KASP Assay Mix can be thawed and re-frozen without affecting its performance, but it is advised not to do this more than three additional times (excluding any freeze-thaw during transit). It is best practice to make smaller aliquots of the assay that can be thawed as required. Once thawed, it is acceptable to store the KASP Assay Mix in the fridge for up to 14 days.

Please note: If KASP Assay mix is shipped alongside KASP-TF Master Mix, it may arrive frozen.

KASP-TF Master Mix

KASP-TF Master Mix should be kept cool and frozen during shipment if possible. KASP-TF Master Mix that arrives at or below 20 °C will be stable and should be frozen upon receipt. We have shown that thawing during transit will not adversely affect performance. Please note that KASP-TF Master Mix should not be stored at greater than 20 °C for more than 48 hours during transit. It is important that KASP-TF Master Mix is frozen immediately upon receipt.

When thawed for first use in your laboratory, Biosearch Technologies recommend to aliquot larger volumes of KASP-TF Master Mix into smaller volumes, and to re-freeze these. Once these aliquots are subsequently thawed for use, further freeze-thaws of KASP-TF Master Mix should be avoided as these may have a negative effect on stability. After thawing an aliquot, it can be stored in the fridge for up to 14 days.

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Section 4: Document links

KASP free of charge trial kit	Request a free-of-charge KASP trial kit to run in your laboratory.	
New Customer Account Form	Complete this form if you are a new Biosearch Technologies customer.	
Sequence Submission Form (KBD and KOD)	Complete this form to request design of KBD and KOD Assays. Enter your sequences of interest with the polymorphisms marked.	
KASP Assay Design Factsheet	View how to enter your sequence information in the Sequence Submission Form, and see examples.	
DNA Submission Form (KOD only)	Complete this form to tell us about the DNA samples you will send to Biosearch Technologies for your KOD Assay project. We will use your DNA samples to validate and optimise your KOD Assays. Not necessary for human KOD Assays - see <u>Note A</u> .	
DNA Requirements for KOD Projects	View details on the quantity of DNA required for a KOD Assay project and how to prepare and ship your DNA to Biosearch Technologies.	
KASP Quick Start Guide	Quick start guide to running KASP chemistry.	
KASP User Manual	Full details on KASP, including experimental setup and data analysis.	
KASP Protocols by PCR Instrument	Download or request the KASP protocol for your instrument.	
KASP Troubleshooting Guide	Common causes of unexpected genotyping results.	
How KASP Works	Learn the basics of KASP genotyping chemistry.	
KASP videos	Learn the basics of KASP genotyping chemistry.	
Analysis of KASP genotyping data using cluster plots	Learn how to analyse KASP genotyping data using cluster plots.	

Section 5: Contact information

Order status enquiries:	Pricing and new order enquiries:	Technical enquiries:
Biosearch Technologies KASP Assays Team Hoddesdon +44 1992 470757 <u>assays@lgcgroup.com</u>	Biosearch Technologies Customer Services Team Hoddesdon +44 1992 470757 genomics.americas@lgcgroup.com genomics.emea@lgcgroup.com	Biosearch Technologies Technical Support Team Hoddesdon +44 1992 470757 techsupport@lgcgroup.com

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Section 6: Delivery and payment information

Delivery times:	Standard service for KBD is 2-3 weeks Standard service for KOD is 4-6 weeks; rapid service is 2-3 weeks KASP-TF Master Mix is typically dispatched within 2 working days.
Delivery format:	KASP Assay Mixes are supplied in 2D barcoded tubes with additional printed labels and are shipped at ambient temperature. KASP-TF Master Mix is supplied in light-protective bottles and is shipped frozen.
Payment conditions:	30 days after date of invoice net and please note in some instances we may request full payment in advance.

All prices are net. They do not include shipping costs and taxes where applicable. As VAT on services accrues to the country where the customer resides, the customer has to account for the VAT and needs to provide the VAT identification number to Biosearch Technologies for correct invoicing.

All services offered are subject to LGC's standard terms and conditions.



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