Guide

DNA requirements for laboratory-validated KASP assays (KASP-On-Demand)

For submission of sequence information

BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Guide

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For laboratory-validation of human KASP-on-Demand (KOD) assays, LGC Biosearch Technologies will use an in-house panel of 44 human DNA samples. For validation of KOD assays for any species other than human, Biosearch Technologies requires a set of DNA samples from the customer. This document outlines the DNA requirements for in-house laboratory-validation of KOD assays. Biosearch Technologies requires sufficient DNA to enable each KASP Assay to be run several times; this allows for assay optimisations to be performed and alternative assay designs to be run where necessary.

Section 1: Outlines the general guidelines for preparing DNA samples for a KOD assay validation project.

Section 2: Details the DNA requirements for an assay validation project.

Section 3: Provides general information on preparing your plates for shipping to Biosearch Technologies.

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Validation of KASP Assays for human samples

When ordering KASP Assays that are to be validated on human samples, it is not necessary to provide DNA samples. Biosearch Technologies has a human validation panel consisting of 44 DNA samples. Unless otherwise specified, this panel will be used for laboratory-validation of KASP Assays for human SNPs or indels. If you would prefer to send in your own human DNA samples for assay validation, please follow the guidelines in this document. Please be aware that if your assay is designed to detect a rare allele (minor allele frequency, MAF) with a MAF <0.0335, it will be beneficial to send in positive controls of the minor allele. In the absence of positive controls, you will need to accept monomorphic validation data.

Section 1: General requirements for DNA sample submissions

Please follow the guidelines in table 1.1 below when preparing your DNA for an assay validation project.

DNA samples – wet or dry?	DNA samples can be shipped to Biosearch Technologies in a dried down or liquid format. DNA requirements are outlined in both mass and concentration in Section 2.
Plate format	DNA samples must be shipped to Biosearch Technologies in 96-well plate format. All the DNA samples for one project must be shipped in one single plate.
Sample number	Biosearch Technologies requires a minimum of 22 DNA samples for assay validation to facilitate cluster analysis. For KOD validation, up to 92 DNA samples can be sent (+4 empty wells required as no template controls, NTC).
DNA extraction method	All DNA samples sent to Biosearch Technologies should be extracted and prepared using the same method.
Elution buffer	Please avoid the use of EDTA in DNA elution buffer as EDTA interferes with the PCR reaction. Where the use of EDTA cannot be avoided, please state the amount of EDTA present in your samples.
Normalised DNA	All samples within the plate must meet the minimum requirements (mass or concentration) for your project. As far as practicably possible, please ensure that the mass or concentration of DNA across your sample plate is consistent, but a variation of 3 to 5-fold (above minimum requirement) across the plate can be accepted.
DNA quality	DNA should be of good quality – a general rule is that if it is possible to PCR the DNA, then it will be suitable for KASP.
DNA quantification method	A range of methods can be used for quantification of DNA samples, including spectrophotometry and PicoGreen. Spectrophotometers have a tendency to overestimate the quantity of DNA present whilst PicoGreen is more exact. Please view our factsheet for more information. All DNA quantities/concentrations in this document assume that samples have been quantified using spectrophotometry.
Dry DNA (mass)	Please note that the mass of DNA required per sample per SNP will differ depending on the genome size of your study organism. If you are working with human DNA (genome size ~3000 Mbp), we require 30 ng good quality DNA per sample per KASP Assay (SNP or indel). Please see table 2.1 for a general guide to the amount of DNA required when sending in dried down samples.

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Wet DNA (concentration and volume)	Please note that the concentration of DNA required will vary based upon the genome size of your study organism. The minimum concentration that we accept is 5 ng/µL. Please see table 3.1 for a general guide to the concentration of DNA required when sending in wet samples. For wet DNA, it is important to send a sufficient volume of DNA for the number of assays being validated, at the appropriate concentration for your species. Table 3.2 outlines the volumes of DNA to send. The minimum acceptable volume is 30 µL per sample. The volume of DNA supplied must be consistent across the sample plate (i.e. same volume for each sample).
Known genotypes	Within your DNA sample plate, please include samples of known genotype as this increases options for assay optimisation. At least one sample of each genotype (homozygous for allele X, heterozygous, or homozygous for allele Y) should be included and identified in your plate map file. This enables Biosearch Technologies to check that all three genotypes are being properly detected, before classifying an assay as validated. Please provide Biosearch Technologies with information regarding the location and expected genotypes (for each assay) of your positive controls within your DNA sample plate(s). This can be provided in a table format (e.g. MS Excel), similar to the example below. Please ensure that the assay names exactly match the names supplied in your SNP submission form, and that the plate and sample names exactly match information supplied in your plate map file.

	A	B	C	D	E	F	G
1	Plate	Well	SampleID	Example_Assay_101	Example_Assay_102	Example_Assay_103	Example_Assay_104
2	DNA_PLATE01	A01	ctrl-2001	T:T	G:G	A:A	A:A
3	DNA_PLATE01	B01	ctrl-2002	C:T	G:G	A:A	A:A
4	DNA_PLATE01	C01	ctrl-2003	C:C	T:T	A:A	G:G
5	DNA_PLATE01	A12	ctrl-3001	C:C	G:T	C:C	A:G
6	DNA_PLATE01	B12	ctrl-3002	C:C	G:G	A:C	A:A
7	DNA_PLATE01	C12	ctrl-3003	?	?	?	A:A

Table 1.1. General guidelines for preparing DNA samples for an Biosearch Technologies assay validation project.

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Section 2: DNA requirements: mass and concentration

Please see table 2.1 below for a general guide to the amount of DNA required per sample per KASP Assay when sending in DNA samples for KOD assay validation project. The amount of DNA required varies according to genome size; more DNA is required for species with larger genomes to ensure that sufficient copies of the genome are present at the start of the KASP reaction. Values are given in mass for dry DNA samples and as a volume and concentration for wet DNA samples.

Genome size	Minimum DNA requirement (per sample per KASP Assay)		Example species
	Mass (dry DNA)	Volume and concentration (wet DNA)	
100-750 Mbp	30 ng	6 µL at 5 ng/µL	*Oryza sativa* (rice), *Arabidopsis thaliana, Drosophila melanogaster* (fruit fly)
751-2000 Mbp	30 ng	6 µL at 5 ng/µL	*Solanum tuberosum* (potato), *Glycine max* (soybean), *Danio rerio* (zebrafish)
2001-3500 Mbp	30 ng	6 µL at 5 ng/µL	*Homo sapiens* (human), *Zea mays* (maize/ corn), *Helianthus annuus* (sunflower), *Mus musculus* (house mouse),
3501-5000 Mbp	48 ng	6 µL at 11 ng/µL	*Lens culinaris* (lentil)
5001-10000 Mbp	99 ng	6 µL at 22 ng/µL	*Hordeum vulgare* (barley)
10001-15000 Mbp	150 ng	6 µL at 33 ng/µL	*Vicia faba* (Faba bean/broad bean)
15001-20000 Mbp	198 ng	6 µL at 44 ng/µL	*Allium cepa* (onion)
20001-30000 Mbp	300 ng	6 µL at 67 ng/µL	*Pinus cembra* (pine)
30001-50000 Mbp	498 ng	6 µL at 111 ng/µL	*Lilium candidum* (Madonna lily)

Table 2.1. Guide to the amount of DNA required per sample per KASP Assay, based on the genome size of the study organism. Values are given as mass and as volume and concentration to account for dry and wet DNA samples respectively.

When sending wet DNA samples, Biosearch Technologies requires a **minimum of 6 µL DNA per sample per KASP Assay** at an appropriate concentration for the genome size of your study organism.

Due to laboratory handling procedures, **we do not accept volumes of less than 30 µL for a KOD assay validation project**. If you are only having a small number of KASP Assays validated, you will still need to supply 30 µL of starting material. The volume supplied per sample must be consistent across the plate.

When sending dry DNA for validation of small numbers of KASP Assays, we require a minimum mass that can be hydrated to give a volume of no less than 30 µL DNA at the working concentration for your organism (see table 2.1).

Worked example: Dry DNA

If your project is for the validation of 6 *Mus musculus* (house mouse) assays, the mass of DNA should be calculated as follows:

30 ng DNA per KASP assay × 6 KASP assays = 180 ng DNA per sample.

Worked example: Wet DNA

If your project is for the validation of 2 *Helianthus annuus* (sunflower) assays, DNA should be prepared at a concentration of 5 ng/µL. The volume of DNA should be calculated as follows:

6 µL DNA per KASP assay × 2 KASP assays = 12 µL DNA per sample

As the minimum acceptable volume for a KOD assay validation project is 30 µL, the DNA requirement for this project is 30 µL DNA per sample at 5 ng/µL.

Section 3: Preparing your plates for shipping

Plate format and labelling

DNA samples must be submitted in a 96-well plate format. All DNA plates that are sent to Biosearch Technologies must be labelled with your individual project number; this number is assigned by Biosearch Technologies and will be in the format 1234.123. If you have not received a project number, please contact assays@lgcgroup.com before shipping your samples to Biosearch Technologies. Your sample plate should also be labelled with an appropriate plate ID. The plate ID on the physical plate should match the plate ID contained in your completed plate map file (see below).

Completion of a plate map file

Prior to shipping the DNA plate, a plate map file must be completed and sent to the assays team (assays@lgcgroup.com). Plate map files can be accessed here. This completed file can be imported directly into our Kraken database and enables your sample IDs to be tracked throughout our laboratory. Without a completed plate map file, Biosearch Technologies will not be able to commence work on your KOD assay validation project.

Please also print a copy of your completed plate map file and include this in the package when shipping your sample plate to Biosearch Technologies.

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Provision of expected genotypes information

If you do not provide Biosearch Technologies with the expected genotypes of your samples (if known), this may result in a delay to the completion of your project. If genotypes are unknown, please advise the assays team at the start of your project.

Plate sealing

Plates should be securely sealed. Biosearch Technologies recommends the use of a heat seal or of cap mats/cap strips to ensure that each well is individually sealed. Sticky seals do not provide a sufficient seal to prevent leakage during transit.

Shipping conditions

DNA sample plates containing wet DNA should be shipped on wet or dry ice to keep samples cool during transit. If you are sending dried down DNA, this is stable and can be shipped at ambient temperature.

Shipping address

Please send KOD assay validation project plates to the following address, include your project number on the front of the package and a printed copy of your plate map file within the package:

LGC Biosearch Technologies
Attention: KOD team, Project [Insert project number e.g. 1234.123]
Unit 27 Trident Industrial Estate
Pindar Road
Hoddesdon, Herts
EN11 0DE
UK

If required, Biosearch Technologies can return your DNA plate to you; you will, however, be required to arrange the return shipping. After completion of a KOD validation project, any remaining DNA will be destroyed unless otherwise requested.

Further support

If you require additional support, please contact our technical support team at techsupport@lgcgroup.com.

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