MRC Holland Support

What are the sample and buffer requirements for (digital)MLPA?

The main sample requirements for MLPA and digitalMLPA are listed below.

Note
The instructions for individual probemixes may differ; always read the probemix-specific product description for more details. For more on sample treatment and quality, please read the instructions within the (MS-)MLPA General Protocol or the digitalMLPA General Protocol.

Amount

A conventional MLPA reaction requires a total quantity of 50–250 ng of DNA in a 5 µl volume (50–100 ng is optimal). A digitalMLPA reaction requires 20–250 ng in a 4 µl volume (40–100 ng is optimal). The instructions for some products may differ.

The DNA sample volume should never be increased above the stated volume (5 µl for conventional MLPA; 4 µl for digitalMLPA). This reduces the concentrations of probes and salts, which in turn negatively affects the hybridisation efficiency and stability.

DNA concentration measurements based on optical density (260 nm) tend to overestimate DNA concentrations, e.g. due to the presence of RNA. Whether sufficient DNA was added can be estimated based on the signals of the Q-fragments in a conventional MLPA experiment. For more information about the Q-fragments and how to interpret them, see this e-learning module. Coffalyser.Net™ for conventional MLPA and Coffalyser digitalMLPA™ for digitalMLPA will provide a warning if there was insufficient sample DNA present.

Buffer

We recommend dissolving and diluting samples in $TE_{0.1}$ (10 mM Tris-HCl pH 8.0 + 0.1 mM EDTA).

It is important that the DNA sample contains 5–10 mM Tris-HCl buffer with a pH of 8.0–8.5 to prevent depurination. For conventional MLPA experiments, we recommend adding 1 µl of 50 mM Tris-HCl pH 8.5 to 4 µl of sample DNA if it is unknown if sufficient buffering capacity is present.

Higher concentrations of EDTA in the TE buffer should be avoided, as this may lead to reaction failure. Conventional MLPA experiments are more sensitive to the EDTA concentration than digitalMLPA experiments.

Never dissolve or dilute samples in water, as it lacks sufficient buffering capacity. In addition, do not use PCR buffer, as it contains salts that can hamper denaturation.

Tissue source

Suitable DNA sample origin varies between probemixes. Always consult the relevant instructions for use for suitable tissue sources. Examples of tissue sources that are suitable for some products are: blood, buccal swabs, fresh healthy/tumour tissue, or FFPE healthy/tumour tissue.

Purity

Samples should be free from impurities known to affect (digital)MLPA reactions. See this article for more details.

Tags

digitalMLPA

MLPA

Related Pages

- How should I store DNA samples?
- Is there a specific DNA extraction method I need to use for (digital)MLPA samples?
- Ethanol precipitation protocol
- Is it possible to use DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissue for (digital)MLPA?
- Is the purity and quality of the sample DNA important for (digital)MLPA?
- What control samples should be included in (digital)MLPA experiments?

Disclaimer