Validation Summary: New MLPA Reagents (2019)

Introduction

From March 2019 onwards, all shipments of SALSA MLPA Reagent Kits include a modified SALSA MLPA Buffer. There has also been a small change in the storage buffer of the SALSA Ligase-65 enzyme.

Changes

The new MLPA buffer contains the antioxidant dithiothreitol (DTT). This counters sloping (the effect that the peak height decreases with increasing probe length) caused by iron ions in the sample DNA, which is a common impurity in DNA samples extracted by magnetic bead-based systems.

The Ligase-65 enzyme remains unchanged, but the β-mercaptoethanol antioxidant in its storage buffer has been replaced by DTT because this is less pungent and less toxic.

Background

We noticed that iron-induced sloping has become a more frequent issue due to the popularity of magnetic bead-based extraction systems. In 2015, we introduced the S4 Sample Stabiliser to reduce this type of sloping. Customer feedback confirmed that the S4 Sample Stabiliser greatly reduces, or completely eliminates, iron-induced sloping. With the addition of one of the active ingredients of the S4 Sample Stabiliser (DTT) to the MLPA buffer, this optimization will be freely available to all our customers without the need for an extra pipetting step. The new MLPA buffer can also be used together with probemixes registered for in vitro diagnostic use, while the S4 Sample Stabiliser is for research use only.

Validation

The new reagents have been tested extensively by MRC-Holland and external laboratories, both on samples extracted by bead-based methods and non-bead based methods under various conditions and with multiple probemixes. No adverse effects were found.

Results of the internal and external tests with the new reagents showed the following:

- **Effectiveness:**
  - Sloping reduction in the presence of 50 µM Fe.
  - Sloping reduction on samples extracted with a magnetic bead-based system.
  - Similar level of sloping reduction in the presence of iron ions as with the old reagents + S4 Sample Stabiliser.

- **Variability:**
  - No effect on the standard deviation of probes on normal samples.
  - No effect on the robustness of results in combination with several common pipetting errors.
  - No effect on the denaturation of GC-rich areas of the sample DNA.
  - No effect on Ligase-65 or polymerase activity.

- **Stability:**
  - No effect on the accelerated aging of MLPA buffer or Ligase-65 enzyme at 30°C.
  - Stable when exposed to up to 25 freeze-thaw cycles.

- **Alternative protocols:**
  - No effect on digestion with HhaI in MS-MLPA.
  - No effect on results in combination with the denaturation buffer used with SALSA MLPA Probemix ME029 FMR1/AFF2.
  - No effect on results with the alternative protocol used for SALSA MLPA Probemix P520 MPN mix 2.

Examples

An example of the effect of the new buffer is shown in Figure 1. The same DNA sample, extracted with a system based on magnetic beads, was used for MLPA reactions with the old and the new reagents, and with or without the S4 Sample Stabiliser. The new reagents reduce sloping (compare panels A and C), and do so

as efficiently as the old reagents with the S4 Sample Stabiliser (compare panels B and C). Adding the S4 Sample Stabiliser to the new reagents has no additional positive effect (compare panels C and D).

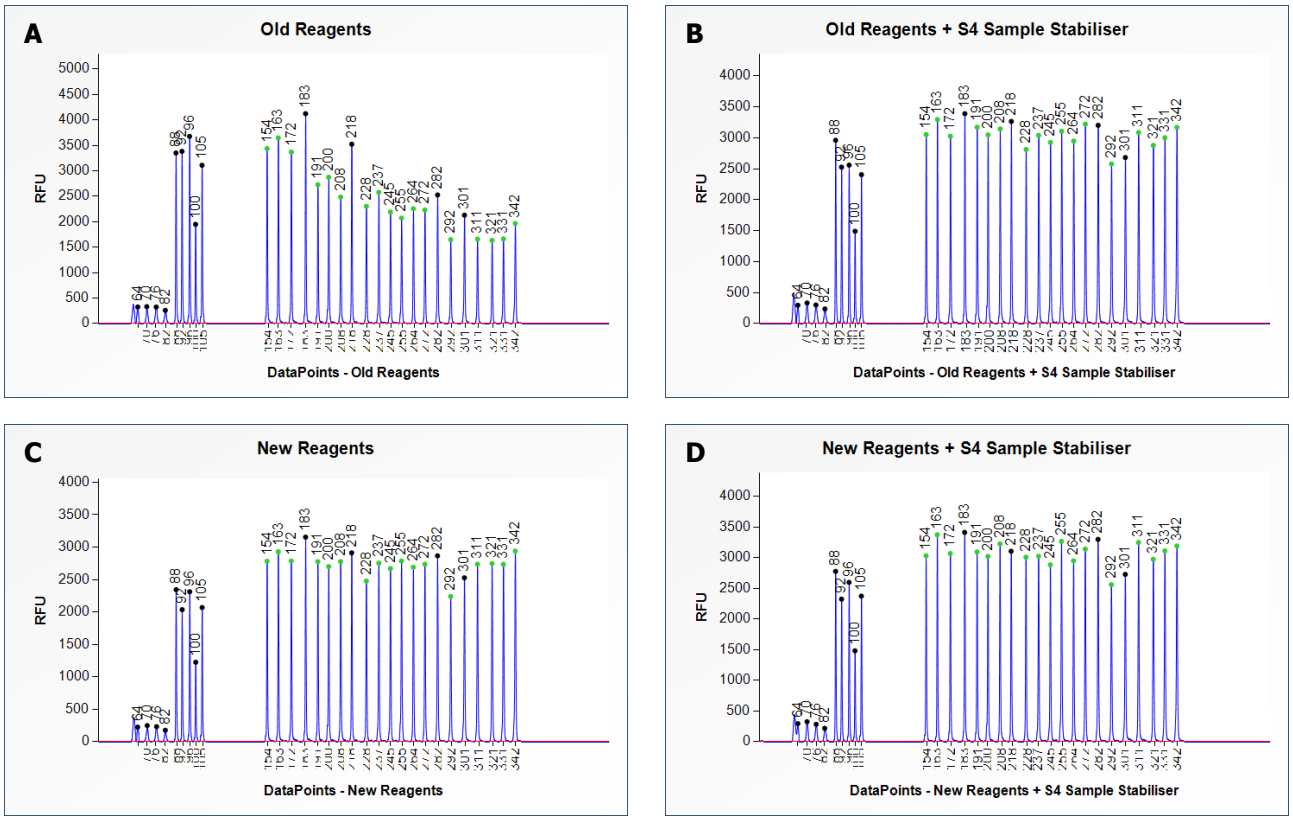

Figure 1. Electropherograms from reactions on the same DNA sample, extracted with a system based on magnetic beads. **A.** Using the old MLPA buffer and Ligase-65 solution. **B.** Using the old MLPA buffer and Ligase-65 solution and the S4 Sample Stabiliser. **C.** Using the new MLPA buffer and Ligase-65 solution. **D.** Using the new MLPA buffer and Ligase-65 and the S4 Sample Stabiliser.

Coffalyser.Net calculates the amount of signal sloping as the internal sloping percentage. Figure 2 shows a summary of the obtained internal sloping percentage of 23 different samples within a representative experiment. The new reagents reduced sloping as effectively as the old reagents with the S4 Sample Stabiliser.

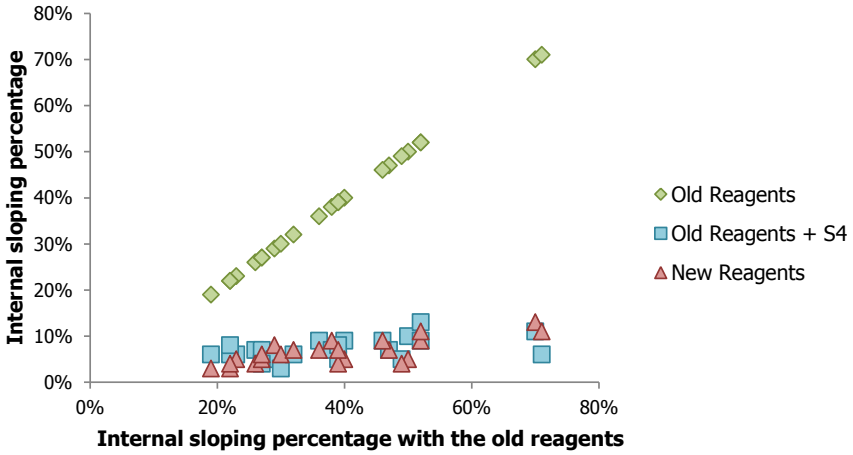

Figure 2. Internal sloping percentage of different DNA samples extracted with a system based on magnetic beads. Reactions using the old reagents (◆), using the old reagents with the S4 Sample Stabiliser (■), and using the new reagents (▲) were performed. The resulting internal sloping percentage of each reaction is shown on the y-axis, plotted against the internal sloping percentage using the old reagents (without the S4 Sample Stabiliser) on the x-axis.

Important Remarks

The new reagents only reduce iron-induced signal sloping. Other causes of signal sloping are not addressed. To reduce variation, it remains critically important to ensure that all samples within an experiment are treated as similarly as possible.

The S4 Sample Stabiliser contains additional active ingredients and will remain available. The other S4 ingredients can prevent problems during sample DNA denaturation but are expected to be beneficial only for a limited number of customers. The MLPA buffer must be added *after* sample DNA denaturation due to its high salt content. Therefore, it was not useful to include the additional S4 ingredients in the new MLPA buffer.

For questions about the new reagents, the S4 Sample Stabiliser, or other sloping issues, please contact our Technical Support department via this form or at info@mlpa.com.

For questions about ordering and shipments, please contact our Sales Support department at order@mlpa.com.