お客様各位

平成 25 年 1 月 8 日

MLPA buffer リニューアルのお知らせ

謹啓　時下ますますご清栄のこととお慶び申し上げます。平素は MLPA キットをご愛顧賜りまして、厚く御礼を申し上げます。

この度、MLPA buffer（黄色キャップの試薬）がリニューアルされることとなりましたので、ご案内を申し上げます。現行品と比較して、下記利点が期待できます。なお、本リニューアルに伴う実験プロトコルの変更はございません。

〈新 MLPA buffer による主な利点〉

・各プローブの PCR 反応が効率化し、データのバラツキを小さくできます。
・MLPA buffer ミクスチャーの添加量の誤差や、PCR 反応時の蒸発、サーマルサイクラーの温度傾斜速度の違いによるデータへの影響が軽減されます。
・Methylation-specific MLPA(MS-MLPA)については、特に結果の改善が期待できます。
※MRC-Holland 社の検証結果は別紙をご参照ください。

新しい MLPA buffer への切替につきましては、誠に勝手ながら、下記のスケジュールにて段階的に実施させていただきます。皆様にはご迷惑をおかけいたしますが、何卒ご理解を賜りますようお願い申し上げます。

本件に関しまして、ご不明な点がございましたら、ご遠慮なくお問合せをお願い申し上げます。

MRC-Holland 社ならびに弊社は、これからも MLPA 試薬の更なる充実を図る所存でございますので、今後とも変わらぬご厚誼を賜りますよう、重ねてよろしくお願い申し上げます。

謹白

-記-

MLPA buffer 切替スケジュール

ご注文受付日	従来の MLPAbuffer	新 MLPA buffer
2013 年 3 月 21 日迄	○	試供
2013 年 3 月 22 日～2014 年 7 月頃(予定)	△	○
2014 年 7 月 以降（予定）	×	○

○・・・基本試薬のお取り扱いとなります。
試供・・・1 キットのご注文につき、無償で 1 本(100 反応分)を商品に同梱させていただきます。
△・・・ご要望をいただいた場合のみ手配させていただきます。
×・・・販売を終了させていただきます。

以上

株式会社ファルコバイオシステムズ
バイオ事業推進部　遺伝子営業課
〒613-0036 京都府久世郡久御山町田井西荒見 17-1
TEL: 0774-46-2639　FAX: 0774-46-2655
E-mail: contact@falco-genetics.com

Background on the introduction of a new MLPA buffer in January 2013.

At MRC-Holland, we routinely test the effect of many variables on MLPA reactions. During these tests, we noticed that pipetting accuracy of the probemix-MLPA buffer mixture has a large influence on the standard variation of certain MLPA probes, which can make MLPA results more variable. This is due to the fact that nearly all salt ions required for the PCR reaction are present in the MLPA buffer.

We investigated the reason why, even though all probes use the same PCR primer pair, some probes are more sensitive to the salt concentration in the PCR reaction than others. Our experiments showed that a sequence in our phage M13-derived probes can form a hairpin. This hairpin formation is not only salt dependent, but also probe dependent and therefore has a large influence on the results obtained. The effects of this hairpin also proved to be dependent on the thermocycler used, in particular on its ramping speed. Our research showed that these effects can virtually be eliminated by the inclusion of a blocking oligonucleotide in the MLPA buffer which prevents this hairpin formation.

The main advantages of the new MLPA buffer as shown in our experiments:

1. All MLPA probemixes show a reduced standard variation of the probes. The beneficial effect of the new buffer depends on the probemix tested, but during our extensive testing, we did not identify a single product whose performance deteriorated with the new MLPA buffer.
2. The number of abnormal results due to pipetting errors of the MLPA buffer is reduced considerably (Table 1).

Table 1. The effects of deliberate pipetting mistakes in 52 different MLPA probe mixes. Instead of 3 µl of the probemix-MLPA buffer mixture, only 2.4 µl was added to a normal DNA sample. For the reference samples, the normal 3 µl of the probemix-MLPA buffer mixture was used. Ratio that should have been obtained in this sample is 1.0; below is shown the frequency of finding strongly deviating ratios.

ABNORMAL RESULTS	Old MLPA buffer	New MLPA buffer
Reactions with at least one probe ratio < 0.75	16 %	0%
Reactions with at least one probe ratio < 0.80	32 %	12 %
Reactions with at least one probe ratio > 1.25	64 %	8 %
Reactions with at least one probe ratio > 1.35	40 %	2 %

Although a 20% pipetting mistake may seem quite large, it is not unusual for substantial pipetting errors to occur when pipetting small volumes.

3. Results of MS-MLPA experiments show a dramatic improvement

Not only did the hairpin formation cause variability in copy number MLPA, the hairpin formation also proved to be a major cause of variable results of the undigested probes in MS-MLPA reactions. As the undigested probes are used for calculating methylation ratios, the results for MS-MLPA analysis improve dramatically by using the new MLPA buffer. As a typical example, results are shown in Tables 2 and 3 for the digested reactions on 16 normal samples tested with ME030-C1.

Table 2. Probes with an expected ratio of 1.00 (digested reactions; 30 probes without a Hha1 site).

MS-MLPA ABNORMAL RESULTS	Old MLPA buffer	New MLPA buffer
# probes with a ratio > 1.4 in at least one of the 16 reactions:	7	0
# probes with a ratio > 1.2 in at least one of the 16 reactions:	15	3

Table 3. Probes with an expected value of 0.50 (digested reactions, 8 MS-MLPA probes in imprinted region with a HhaI site); tested on 16 different samples.

MS-MLPA ABNORMAL RESULTS	Old MLPA buffer	New MLPA buffer
# probes with a ratio <0.4 in at least one sample	2	0
# probes with a ratio >0.6 in at least one sample	7	1
Lowest probe ratio observed (in 16 samples)	0.36	0.42
Highest probe ratio observed (in 16 samples)	0.75	0.62

Disadvantages of the new MLPA buffer:

Although the new MLPA buffer has major advantages, unfortunately, it also has a few minuses.

1. For some existing probemix lots, the use of new MLPA buffer increases the height difference between the lowest and highest MLPA peaks, increasing the likelihood that one or more high peaks will go off-scale. All our newly produced probemix lots will be optimized for use with the new MLPA buffer.
2. We found a small number of products with a reproducible peak in the No DNA reactions that slightly increased in peak height with the new buffer. In none of the cases we have evidence that results of MLPA experiments are affected in any way by this.

The feedback provided by more than 25 different customers who tested the new MLPA buffer on October 2012 on average confirmed the conclusions from our experiments.

If you have questions about the introduction of the new MLPA buffer, please do not hesitate to contact our technical support staff at info@mlpa.com or by calling +31 20 4124731. For questions about ordering the new MLPA buffer, please contact our sales department (e-mail: order@mlpa.com, phone: +31 20 4891248).

Best regards,
Jan Schouten
CEO of MRC-Holland b.v.