STANDARD PROGRAMS FOR MOSQUITO (except LCP*)

Classic protocols (full plate, 1 or 2 drop, 1:1:0 ratio)

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Name	P:R:A (nl)	Stripes number and type	Vol. well (ul)	vol. tot. (ul)
MRC plate 50 nl 1 drop	50:50:0	2ul (pos. 1)	1.4	12
MRC plate 50 nl 2 drop	50:50:0	2ul x 2 (pos. 1 & 2)	1.4	24
MRC plate 100 nl 1 drop	100:100:0	2ul (pos. 1)	2.0	16
MRC plate 100 nl 1 drop R first	100:100:0	2ul (pos. 1)	2.0	16
MRC plate 100 nl 2 drop	100:100:0	2ul x 2 (pos. 1 & 2)	2.0	16 x 2
MRC plate 200 nl 1 drop	200:200:0	5ul (pos. 1)	3.8	30
MRC plate 200 nl 2 drop	200:200:0	5ul x 2 (pos. 1 & 2)	3.8	30 x 2
MRC plate 500 nl 1 drop	500:500:0	5ul (pos. 1)	7.4	60
MRC plate 500 nl 2 drop	500:500:0	5ul x 2 (pos. 1 & 2)	7.4	60 x 2
MRC plate 1000 nl 1 drop	1000:1000:0	5ul x 2 (pos. 1 & 2)	7.4	120
MRC plate 1000 nl 2 drop	1000:1000:0	5ul x 4 (pos. 1-4)	7.4	120 x 2

P:R:A means Protein:Reagent:Additive ratio

Protocols with different ratios

Name	P:R:A (nl)	Stripes number and type	Vol. well (ul)	Vol. tot (ul)
MRC plate 200 nl 1 drop PR ratio 1to3	100:300:0	2ul (pos. 1)	2.0	16
MRC plate 200 nl 1 drop PR ratio 3to1	300:100:0	5ul (pos. 1)	5.0	40
MRC plate 200 nl 2 drop PR ratio 1to3 3to1	100:300:0;300:100:0	2 ul (pos. 1) + 5ul (pos. 2)	2.0, 5.0	60

Half a plate protocols

Name	P:R:A (nl)	Stripes number and type	Vol. well (ul)	Vol. tot (ul)
MRC plate 1st Half only 100 nl 1 drop	100:100:0	2ul (pos. 1)	1.4	12
MRC plate 1st Half and 2nd Half 100 nl 1 drop	100:100:0	2ul x 2 (pos. 1 & 2)	1.4	24

Additive protocols

Name	P:R:A (nl)	Stripes number and type	Vol. well (ul)	Vol. tot (ul)
MRC plate 200 nl 1 drop + 100 nl A from stripe	200:200:100	5ul (P:pos.1) + 2ul (A:pos.2)	3.8 (P), 2.0 (A)	30 (P), 16 (A)
MRC plate 200 nl 1 drop + 100 nl A from Costar plate	200:200:100	5ul (pos. 1)	3.8	30

MAXI protocols (can be 1 drop only!)

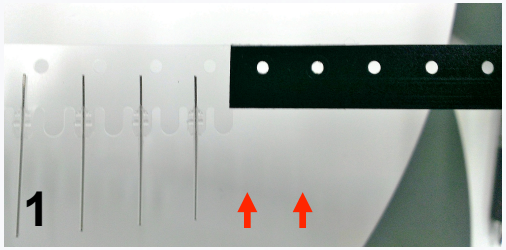
Name	P:R:A (nl)	Stripes number and type	Vol. well (ul)	Vol. tot (ul)
MAXI plate 500 nl	500:500:0	5ul (pos. 1)	4.4	36
MAXI plate 1000 nl	1000:1000:0	5ul (pos. 1)	7.4	60
MAXI plate 1000 nl R first	1000:1000:0	5ul (pos. 1)	7.4	60
MAXI plate 500 nl + 250 nl A from stripe	500:500:250	5ul (P:pos.1) + 2ul (A:pos.2)	4.4 (P), 2.5 (A)	36 (P), 20 (A)
Add 100 nl A from Costar V shape to drops	0:0:100	no	n/a	n/a

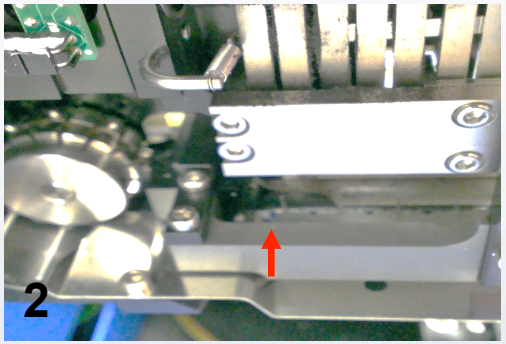
> Standard dead volumes for the wells containing the protein:
0.8ul for 2ul-stripe (contains 3.2ul max.); 1.4 ul for 5ul-stripe (7.5ul max.); 2.5 ul for Costar plate (V-shaped wells).
Dead volume may be higher when Mosquito tips return several times into the wells during a process.

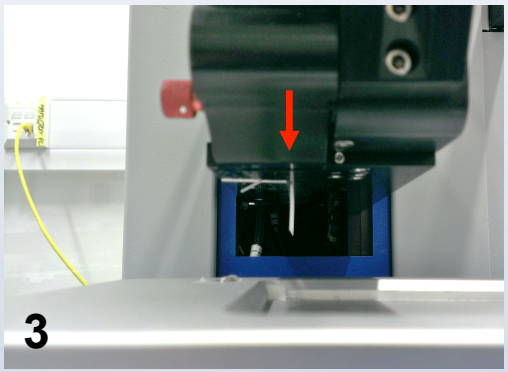
> First stripe always in column 1 of the holder, second stripe in column 2, etc.

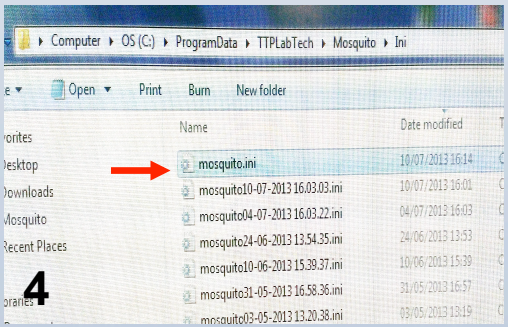
> Protocol "R first" means reagents are dispensed first (case where the protein accumulates at the end of the tips)

Tips for Mosquito Tips (“The Phase problem”)









Reloading a spool. When things go wrong (especially the phase), the best thing to do is probably to proceed with reloading the spool. For that you need to place a spare black-leader-tape on the spool: make sure you cut the side of the tape along with the first couple of tips (Figure 1).

Calibrating the Phase: The first tip at the back of the head must be aligned with the first groove of the clamp (Figure 2). The phase is right when tips form a straight line and are perpendicular to the deck with the clamp closed (Figure 3). If the tips can never be relatively well-perpendicular to the deck with the clamp closed, the spool is faulty and may cause problem later.

Bug with the ini file: When calibrating the phase, if you jogged the spool further than 9 mm, the “ini” file get corrupted (Figure 4). This file, called *mosquito.ini*, contains various parameters used for the instrument (including the new calibration parameters). Replace the corrupted file by the former ini file (i.e. switch file names), hence the former file will be taken up by the instrument. Then proceed with calibration again.