Bench sheet · EnGeL-MOLB-KAPA

Illumina library quantification by qPCR (KAPA Library Quantification Kit) · version 1.2.0 (2026-11-20). Full protocol: EnGeL-MOLB-KAPA. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
KAPA Library Quantification Kit lot	

Before starting

☐	No.	Step
☐	•	Clean the working space and material with bleach and water and leave the UV light on for at least 15 min.
☐	•	Use filter tips at all steps.
☐	•	Clean forceps and spatulas with 3% sodium hypochlorite and 96% ethanol between samples.

Quantification

☐	No.	Step
☐	1	Add the 1 mL Primer Mix (10X) to the 5 mL bottle of SYBR FAST Master Mix (2X); vortex briefly.
☐	2	Store the combined mix at −20 °C (or 4 °C for short-term use, per kit insert); keep away from light.
☐	3	Thaw the standards on ice, vortex and spin down. Do not dilute the standards further.

Dilution	Make from	Volume taken (µL)	Dilution buffer (µL)	Use
1:1 000	Pool	1	999	Intermediate only
1:10 000	1:1 000	100	900	Run in qPCR
1:20 000	1:1 000	50	950	Run in qPCR
1:100 000 (optional)	1:10 000	10	90	If PicoGreen > c. 50 nM

☐	No.	Step
☐	4	Prepare the dilutions above. Vortex and spin each dilution before taking the next step. Check against the PicoGreen value: the diluted library should be about 0.2–20 pM, inside the standard range.

Component	Per well (µL)
Master Mix + Primer Mix (combined)	12
PCR-grade water	4
Diluted library, standard or NTC buffer	4

☐	No.	Step
☐	5	Prepare a master mix of 12 µL combined mix + 4 µL water per well, plus 10% excess.
☐	6	Dispense 16 µL per well, then add 4 µL of diluted library, standard or dilution buffer (for the NTC).
☐	7	Seal, spin briefly, and keep the plate in the dark until loading.

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	30 s	35
Annealing/extension + read	60 °C	45 s	
Melt curve	65 °C, then to 95 °C at 0.15 °C/s	15 s at each temperature	1

☐	No.	Step
☐	8	Run the plate on the qPCR instrument (QuantStudio 3) with the cycling above.
☐	9	Export the results as .csv (tab *Export*) and check the run against the run QC criteria before calculating.

After finishing

☐	No.	Step
☐	•	Clean the bench with 70% ethanol.
☐	•	Empty the small bench bin into the right waste bin.
☐	•	Wash your hands.

☐	No.	Step
☐	•	Turn off equipment and lights.
☐	•	Lock doors, fridge and freezer.

Notes / deviations
