



## firefly<sup>®</sup> + technical note

# Roche KAPA EvoPlus Boost Kit and KAPA EvoPlus V2 Kit

This technical note provides supporting information for the firefly+ protocols listed below. These protocols are based on the Roche document - “Instructions for use of KAPA DNA Library Prep Evolved Workflows with enzymatic fragmentation. For use with the KAPA EvoPlus V2 and KAPA EvoPlus Boost kits. Featuring KAPA EvoT4 DNA Ligase and KAPA EvoAmp ReadyMix. (October 2025, Version 5.0)” and are available to download from the firefly community. Here, we outline protocol run times, parts required and provide details on the steps performed in each of the firefly+ protocols.

## firefly+ protocols

### KAPA EvoPlus Boost or KAPA EvoPlus V2 – firefly+

Protocol name	firefly+ run time
3.1-3.3 Fragmentation and Adapter Ligation	1 hour 56 minutes (8 samples)
3.1-4.3a Fragmentation, Adapter Ligation and Amplification	2 hours 54 minutes (8 samples with 5 cycles PCR) to 3 hours 20 minutes (96 samples with 5 cycles PCR)
Appendix A. Double-sided Size Selection	42 minutes (8 samples)
4.1-4.3a Library Amplification and Single Purification	56 minutes (8 samples with 5 cycles PCR)
4.1-4.3b Library Amplification and Double Purification	1 hour 38 minutes (8 samples with 13 cycles PCR)

**Table 1.** firefly+ protocol names and run times. Appendix and Chapter numbers are in reference to the Roche Instructions for Use document.

Workflow	firefly+ protocol to run	
	3.1-3.3 Fragmentation and Adapter Ligation	Appendix A. Double-sided Size Selection
KAPA EvoPlus Boost PCR-Free	✓	
KAPA EvoPlus Boost PCR-Free with post ligation size selection	✓	✓

**Table 2.** Summary of the KAPA EvoPlus Boost PCR-Free workflows that can be run and the firefly+ protocols required to run them. The KAPA EvoPlus V2 PCR-Free workflow can be run as a standalone walkaway protocol or with an additional post-ligation size selection.

Workflow	firefly+ protocol to run				
	3.1-3.3 Fragmentation and Adapter Ligation	3.1-4.3a Fragmentation, Adapter Ligation and Amplification	Appendix A. Double-sided Size Selection	4.1-4.3a Library Amplification and Single Purification	4.1-4.3b Library Amplification and Double Purification
KAPA EvoPlus V2		✓ Using KAPA HiFi HotStart ReadyMix PCR			
KAPA EvoPlus V2 with post ligation size selection	✓		✓	✓ Using KAPA HiFi HotStart ReadyMix PCR	
KAPA EvoPlus V2 with post amplification size selection		✓ Using KAPA HiFi HotStart ReadyMix PCR	✓		
Low input (0.1ng) KAPA EvoPlus V2	✓				✓ Using KAPA HiFi HotStart ReadyMix PCR

**Table 3.** Summary of the KAPA EvoPlus V2 workflows that can be run and the firefly+ protocols required to run them. The KAPA EvoPlus V2 workflow uses the KAPA HiFi HotStart ReadyMix and associated PCR cycling conditions. It can be run standalone or with a post-ligation or post-amplification size selection or with low input (0.1ng) by running the relevant combination of protocols.

Workflow	firefly+ protocol to run				
	3.1-3.3 Fragmentation and Adapter Ligation	3.1-4.3a Fragmentation, Adapter Ligation and Amplification	Appendix A. Double-sided Size Selection	4.1-4.3a Library Amplification and Single Purification	4.1-4.3b Library Amplification and Double Purification
KAPA EvoPlus Boost		✓ Using KAPA EvoAmp ReadyMix PCR			
KAPA EvoPlus Boost with post ligation size selection	✓		✓	✓ Using KAPA EvoAmp ReadyMix PCR	
KAPA EvoPlus Boost with post amplification size selection		✓ Using KAPA EvoAmp ReadyMix PCR	✓		
Low input (0.1ng) KAPA EvoPlus Boost	✓				✓ Using KAPA EvoAmp ReadyMix PCR

**Table 4.** Summary of the KAPA EvoPlus Boost workflows that can be run and the firefly+ protocols required to run them. The KAPA EvoPlus Boost workflow uses the KAPA EvoAmp ReadyMix and associated PCR cycling conditions. It can be run standalone or with a post-ligation or post-amplification size selection or with low input (0.1ng) by running the relevant combination of protocols.

## Protocol Highlights

- Full walkaway library preparation protocol for KAPA EvoPlus Boost (or KAPA EvoPlus V2)
- Full walkaway library preparation protocol for KAPA EvoPlus Boost PCR-Free
- On-deck thermocycler (ODTC) on firefly+ allows all incubation and thermocycling steps to take place on the firefly+
- Flexibility to process 1 to 12 sample columns per run and to specify the starting column for the reagent plates (ReadyMix plates and UDI Adapters plates) - enabling multiple low-throughput runs using the same reagent plates and reducing waste.
- An additional protocol to perform the optional double-sided size selection listed in Appendix A. of the Instructions For Use document. This protocol can be run either at the post-ligation or post-amplification stage
- Additional protocols to run the Library Amplification step and either a single or double post-PCR purification

## Protocol overview

SPT Labtech firefly+ protocols have been developed to run the KAPA EvoPlus Boost, KAPA EvoPlus Boost PCR-Free and KAPA EvoPlus V2 workflows – using the 96-well plate format kits and the KAPA Unique Dual-Indexed (UDI) Adapter Kit. The firefly+ protocols run in 96-well plate format and make use of the on-deck thermocycler (ODTC) on firefly+ to maximise walkaway time. Variables allow a user to run from 1 to 12 columns of library preparation. Either the KAPA EvoPlus Boost or KAPA EvoPlus V2 workflow can be run using the same firefly+ protocols.

For the KAPA EvoPlus Boost workflow, the KAPA EvoPlus Boost Kit (96-well plate) should be used – which contains a KAPA EvoAmp ReadyMix plate. This plate and the KAPA EvoAmp ReadyMix cycling program conditions should be used when selecting the PCR ODTc protocol to run.

For the KAPA EvoPlus V2 workflow, the KAPA EvoPlus V2 Kit (96-well plate) should be used – which contains a KAPA HiFi HotStart ReadyMix (2X) plate. This plate and the KAPA HiFi HotStart ReadyMix cycling program conditions should be used when selecting the PCR ODTc protocol to run.

The KAPA EvoPlus V2 and KAPA EvoPlus Boost workflows on firefly+ cover the following sections of the Instructions for Use document:

### Chapter 3. Prepare the Sample Library:

- Step 1. Fragmentation and A-Tailing
- Step 2. Adapter Ligation
- Step 3. Purify the Sample Library using KAPA HyperPure Beads

### Chapter 4. Amplify the Sample Library:

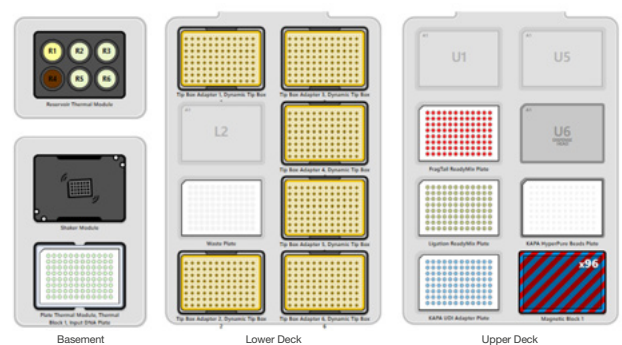
- Step 1. Prepare the Library Amplification Reaction
- Step 2. Perform the Library Amplification
- Step 3. Purify the Amplified Sample Library using KAPA HyperPure Beads

### Appendix A. Double-sided Size Selection

## KAPA EvoPlus Boost PCR-Free workflow firefly+ protocol - 3.1-3.3 Fragmentation and Adapter Ligation

This full walkaway protocol runs the KAPA EvoPlus Boost PCR-Free workflow on firefly+ following Chapter 3. *Prepare the Sample Library of the Instructions For Use document.*

The firefly+ protocol adds the FragTail ReadyMix to input DNA and tip mixes. The input DNA plate is then placed on the On Deck ThermoCycler (ODTC) to run the Fragmentation and A-tailing. Following Fragmentation and A-tailing, the protocol adds KAPA UDI Adapters and Ligation ReadyMix to the FragTail product and tip mixes. The plate is then incubated at 20°C for 15 minutes on the ODTc. Following the Adapter Ligation, the protocol performs a 0.6X bead purification and then transfers the cleaned-up adapter-ligated libraries to a fresh plate.



**Figure 1.** firefly+ starting deck layout for a 96-sample KAPA EvoPlus Boost PCR-Free 3.1-3.3 Fragmentation and Adapter Ligation library prep run. Deck layout – Basement: Reservoir R1 – Elution Buffer, R2+R3+R5+R6 – 80% Ethanol, R4 – KAPA HyperPure Beads, Plate Thermal Module - 96 sample Thermo Adapter Block and Input DNA Plate. Lower Deck: L1+L4+L5+L6+L7+L8 L7 - 100 µL 96 format ATL tips on ATL 35-125 µL Tip Stands, L3 – Waste Plate. Upper Deck: U2 – FragTail ReadyMix Plate, U3 – Ligation ReadyMix Plate, U4 – KAPA UDI Adapter Plate, U7 – KAPA HyperPure Beads Plate, U8 – Alpaqua Magnum FLX 96 sample Magnetic Block.



**Figure 2.** firefly+ starting layout for a 96-sample KAPA EvoPlus Boost PCR-Free 3.1-3.3 Fragmentation and Adapter Ligation library prep run. M1-M3 - 100 µL 96 format ATL tips. S1 – Cleaned up Adapter Ligation Library Plate. S7, S8 – Plate Lids.

## KAPA EvoPlus Boost (or KAPA EvoPlus V2) workflow

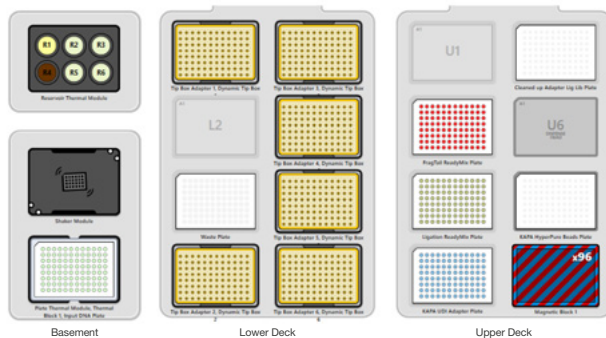
### firefly+ protocol - 3.1-4.3a Fragmentation, Adapter Ligation and Amplification

This full walkaway protocol runs the KAPA EvoPlus Boost (or KAPA EvoPlus V2) workflow on firefly+ following Chapter 3. *Prepare the Sample Library* and Chapter 4: *Amplify the Sample Library* of the Instructions For Use document. This protocol follows Chapter 4, Step 3a *Purification of amplified Sample Library* procedure.

This protocol runs the same initial steps as the PCR-Free protocol and then carries on to the amplification and purification steps.

Following the Adapter Ligation Purification, the protocol adds Library Amplification Primer Mix and KAPA HiFi HotStart ReadyMix (2X) OR KAPA EvoAmp ReadyMix to the Adapter-ligated libraries and tip mixes. The plate is then run on the relevant user defined KAPA Library Amplification program on the ODC. KAPA EvoPlus Boost workflows use the KAPA EvoAmp plate and cycling conditions. KAPA EvoPlus V2 workflows use the KAPA HiFi HotStart ReadyMix (2X) plate and cycling conditions.

Once the thermocycler program is complete, the plate is returned to the firefly deck. The protocol then performs a 1.0X bead purification and transfers the cleaned-up amplified libraries to a fresh plate.



**Figure 3.** firefly+ starting deck layout for a 96-sample KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification library prep run. Deck layout – Basement: Reservoir R1 – Elution Buffer, R2+R3+R5+R6 – 80% Ethanol, R4 – KAPA HyperPure Beads, Plate Thermal Module - 96 sample Thermo Adapter Block and Input DNA Plate. Lower Deck: L1+L4+L5+L6+L7+L8 L7 - 100  $\mu$ L 96 format ATL tips on ATL 35-125  $\mu$ L Tip Stands, L3 – Waste Plate. Upper Deck: U2 – FragTail ReadyMix Plate, U3 – Ligation ReadyMix Plate, U4 – KAPA UDI Adapter Plate, U5 – Cleaned Up Adapter Ligation Library Plate, U7 – KAPA HyperPure Beads Plate, U8 – Alpaqua Magnum FLX 96 sample Magnetic Block.

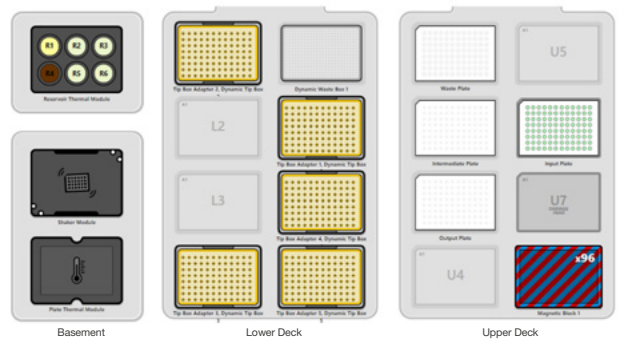
**Figure 4.** firefly+ starting layout for a 96-sample KAPA EvoPlus Boost (or EvoPlusV2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification library prep run. M1-M11 - 100 $\mu$ L 96 format ATL tips. S1 – KAPA Library Amplification Primer Mix Plate, S2 – KAPA EvoAMP OR (2X) HiFi HotStart ReadyMix Plate, S7-S9 – Plate Lids.



## KAPA EvoPlus V2 Double-sided Size Selection workflow

### firefly+ protocol - Appendix A. Double-sided Size Selection

This optional Size Selection protocol can be run after either the firefly+ 3.1-3.3 Fragmentation and Adapter Ligation protocol to perform post-ligation sample purification or after the firefly+ 3.1-4.3a Fragmentation, Adapter Ligation and Amplification protocol to perform a post-amplification sample purification. Input volume is 50  $\mu$ L, and output volume is 20  $\mu$ L. This protocol performs a double-sided Size Selection purification where the user selects the SPRI 1 and SPRI 2 ratios. The size-selected libraries are then transferred to a fresh output plate.



**Figure 5.** firefly+ starting deck layout for a 96-sample KAPA EvoPlus V2 Appendix A. Double-sided Size Selection run. Deck layout – Basement: Reservoir R1 – Elution Buffer, R2+R3+R5+R6 – 80% Ethanol, R4 – KAPA HyperPure Beads. Lower Deck: L1+L4+L6+L7+L8 L7 - 100  $\mu$ L 96 format ATL tips on ATL 35-125  $\mu$ L Tip Stands, L5 – Empty Waste tip Box. Upper Deck: U1 – Waste Plate, U2 - Intermediate Plate, U3 – Output Plate, U6 – Input Plate, U8 – Alpaqua Magnum FLX 96 sample Magnetic Block.



**Figure 6.** firefly+ starting layout for a 96-sample KAPA EvoPlus V2 Appendix A. Double-sided Size Selection run. M1 – Empty Waste tip Box, M2-M3 - 100  $\mu$ L 96 format ATL tips.

## KAPA EvoPlus Boost (or KAPA EvoPlus V2) - Chapter 4. Amplify the Sample Library only

### firefly+ protocols - 4.1-4.3a Library Amplification and Single Purification

#### 4.1-4.3b Library Amplification and Double Purification

These protocols can optionally be run following the 3.1-3.3 Fragmentation and Adapter Ligation protocol to complete the full amplification workflow outlined in Chapter 4. *Amplify the Sample library*, of the Instructions For Use document.

If libraries were constructed from  $\geq 1$  ng of input DNA using KAPA UDI Adapters, then protocol 4.1-4.3a Library Amplification and Single Purification should be run. This will run a single 1.0X bead cleanup post-amplification.

If libraries were constructed from 0.1-1 ng of input DNA using KAPA UDI Adapters, then protocol 4.1-4.3b Library Amplification and Double Purification should be run. This will run two 1.0X bead cleanups post-amplification.

## Reagents

### Kits

Description	Roche Cat No.
KAPA EvoPlus V2 Kit, plated format (96rxn)	09420428001
KAPA EvoPlus Boost Kit, PCR-free, plated format (96rxn)	09420436001
KAPA Library Amp Primer Mix, 96-well plate (96rxn)	09420479001
KAPA EvoPlus Boost Kit, plated format (96rxn)	10613726001
KAPA Unique Dual-Indexed Adapter Kit (15 $\mu$ M)	08861919702
KAPA Adapter Dilution Buffer (25 mL)	08278539001
KAPA HyperPure Beads Kit (60 mL)	8963851001

**Table 5.** Roche KAPA EvoPlus V2 kits required for firefly+ library prep runs.

Reagent volumes for 80% ethanol, KAPA HyperPure Beads, and Elution Buffer (10mM Tris-HCl pH 8.0-8.5) are dependent on the user input variable "Number of Columns". The required volumes of these reagents are shown in the Execute section of the firefly+ software – as shown in Figure 7 below. All other reagents used in this workflow are supplied in plated format, so the user only needs to defrost, vortex, then spin down the reagent plates, pierce the foil, and load them onto firefly+. No master mixes need to be prepared.



**Figure 7.** Example of reagent volumes required for a 12-column KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification run on firefly+.

## Variables

### KAPA EvoPlus Boost (or KAPA EvoPlus V2) workflow

#### firefly+ protocol - 3.1-4.3a Fragmentation, Adapter Ligation and Amplification

Variable	Range
Number of Columns	1-12
UDI starting column	1-12
FragTail starting column	1-12
Ligation starting column	1-12
KAPA EvoAmp OR KAPA HiFi starting column	1-12
Final Elution Volume ( $\mu$ L)	Enter "20" for standard workflow. Enter "50" if proceeding to size selection

**Table 6.** User input variables available for the KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification firefly+ protocol. Appropriate subsets of the set of user input variables are also available for the PCR-Free and Chapter 4 only firefly+ protocols.

ODTC Data files may also be selected by the user for the Frag A-tail, Adapter Ligation, and PCR conditions. Note - the default embedded Frag A-tail ODTC data file is for a Fragmentation time of 20 minutes, and the default embedded PCR cycling conditions in the firefly+ protocols are for the KAPA EvoPlus Boost workflow. Other ODTC data input files are available on request.

### KAPA EvoPlus V2 Double-sided Size Selection workflow

#### firefly+ protocol - Appendix A. Double-sided Size Selection

Variable	Range
Number of Columns	1-12
SPRI 1 ratio	0.4-0.9
SPRI 2 ratio	0.6-1.8 (At least 0.2 higher than SPRI 1 ratio)

**Table 7.** User input variables for KAPA EvoPlus V2 (Appendix A. Double-sided Size Selection).

## Consumables

Supplier	Part name	Part number	Number required	Note
SPT Labtech	Standard Syringe	4150-07200	6	
SPT Labtech	High volume reservoir	4150-07300	6 (total)	Reservoir types needed are dependent on the number of columns processed
	Standard reservoir	4150-07103		
	Low dead volume (LDV) reservoir	4150-07202		
SPT Labtech	100 µL, 96 format, AT-Load Tips (filtered)	125-96-FF-AL-FS	3 to 22 boxes	Number required depends on the number of columns processed
Eppendorf	96-well twin.tec PCR plate	30128648	3	
Thermo Fisher Scientific	96-well Fisherbrand 1 mL Deep Well	236600	1	Waste plate
Hamilton	PCR ComfortLid	814300	3	

**Table 8.** Consumables required for KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification firefly+ runs.

## Parts required for a 96 sample KAPA EvoPrep run on firefly

Supplier	Part name	Part number	Number required	Note
SPT Labtech	ATL 35-125 µL Tip Stand	3276-08335 (REV 2)	3 to 6	Number required depends on the number of columns processed
SPT Labtech	96 Thermo Adapter Block	3276-01065	1	
SPT Labtech	firefly reservoir tray v2	3276-01928	0 to 1	Required for runs of 6+ columns
SPT Labtech	Standard reservoir puck for firefly reservoir tray v2	3276-01897	0 to 2	Number required depends on the number of columns processed
Alpaqua Engineering	Alpaqua Magnum FLX	A000400	1	

**Table 9.** Parts required for KAPA EvoPlus Boost (or KAPA EvoPlus V2) 3.1-4.3a Fragmentation, Adapter Ligation and Amplification firefly+ runs.