Check the product label for actual catalog number, lot and expiry date.

Rally™ Rapid Ligation Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RLK0101	40 r of 20 µl	40 µl – Rally™ T4 DNA Ligase, 1 r/µl 1.5 ml - 2X Rally™ Buffer	Storage buffer contains Tris, 50% glycerol and other components. 2X Rally™ Buffer contains DTT, ATP, PEG 6000 and other components.
RLK0105	200 r of 20 µl	5 x 40 µl – Rally™ T4 DNA Ligase, 1 r/µl 5 x 1.5 ml - 2X Rally™ Buffer	

Storage *In the dark at -20°C.*

APPLICATIONS

- Cloning
- Vector-insert ligation
- Adaptor or linker ligation
- Linear DNA self-circularization

BENEFITS

- 5 minutes fast high efficiency ligation
- Universal for both blunt or cohesive-end ligations
- Premium reagents - reproducible results

PRODUCT DETAILS

Rally™ Rapid Ligation Kit is a premium tool designed for fast and highly efficient ligation reactions, cloning or adaptor/linker joining applications. The Kit contains Rally™ T4 DNA Ligase specially formulated to perform faster; and a 2X buffer which includes PEG to accelerate joining of DNA ends.
The combination of both components allows for an efficient and fast ligation reaction of both blunt and cohesive DNA termini eliminating the need of hours or overnight incubations.

PRODUCT SPECIFICATIONS

- Optimum activity at room temperature, around 25°C
- Inactivation at 65°C for 15 min

Rally™ T4 DNA Ligase, same as the classical enzyme catalyzes the formation of a phosphodiester bond between the terminal 5' phosphate and 3' hydroxyl groups of DNA or RNA. It joins both blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

PROTOCOL

- Check the integrity and the concentration of the DNA prior the ligation.
- Include ligation positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use. If precipitation in the buffer appears, warm it to dissolve.
- Use an insert : vector molar ratio around 3 : 1 to 6 : 1, optimize it if possible. Too much insert causes ligation of multiple inserts, too little reduces ligation efficiency.
- The Rally™ Buffer includes PEG which has almost no effect on transformation efficiency of chemically-competent cells, but reduces the transformation efficiency of electro-competent cells. Therefore, for electrotransformation PEG has to be removed from the mixture or highly diluted.
- Use only high efficiency competent cells for cloning. Check the transformation efficiency of the competent cells by transforming 0.1 ng of supercoiled vector DNA. Getting 100 colonies in this case means you have 1x10^6 transformants/1 µg supercoiled DNA. Take into account that the ligated mixture gives normally at least 10 times less transformants compared to the same amount of supercoiled DNA.

IN VITRO RESEARCH USE ONLY

✓ Prepare a 20 µl reaction:

2X Rally™ Buffer	10 µl
Linear dephosphorylated vector DNA	~100 ng (20 – 200 ng range)
100 ng of linear pUC vectors (~2.7 kb) have ~0.1 pmol ends *100 ng of linear pBR322 vectors (~4.4 kb) have ~0.07 pmol ends*	
Insert DNA (phosphorylated)	~200 ng (up to 3-6 X more pmol ends than vector)
200 ng of 1 kb linear DNA has ~0.6 pmol ends *100 ng of 0.5 kb linear DNA has ~0.6 pmol ends* *40 ng of 0.2 kb linear DNA has ~0.6 pmol ends*	
DNase-free water (PCR Water, WAT0110)	to 19 µl
Rally™ T4 DNA Ligase, 1 r/µl	1 µl maximum

✓ Mix well, incubate for 5-10 min at 25°C (room temperature).
✓ Use directly 1-5 µl of the mixture to transform 50 µl of chemically-competent cells.
✓ For electrotransformation PEG has to be removed from the mixture or diluted as follows:

Option 1: re-purify the ligation mixture using PCR clean-up spin column kit, elute in 20 µl of water or TE and transform 1-2 µl of the eluate into 50 µl of electro-competent cells.

Option 2: immediately after ligation dilute the mixture 10X by adding 180 µl of water or TE and transform 2-5 µl of the diluted mix into 100 µl of electro-competent cells.

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