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DATA SHEET

Version: 03
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1. Identification

Product name

pSpark® Universal

20ng/uL 20 reactions

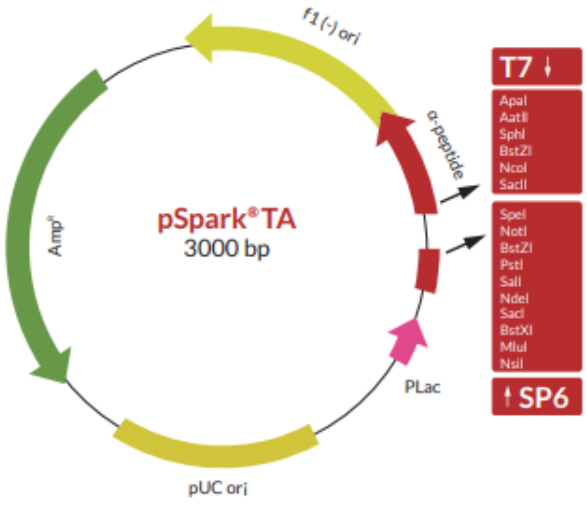
Cat No

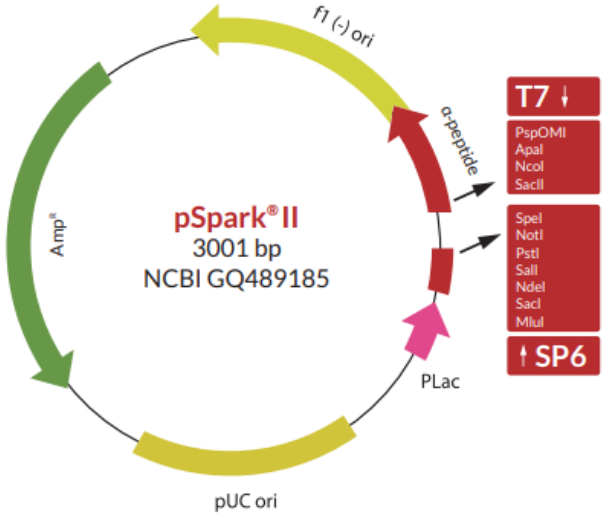
C0019

2. Description

pSpark® Universal is a highly efficient, accurate and easy-to-use DNA Cloning kit ideal for a broad range of PCR fragments cloning applications. There is a range of DNA Polymerases available that do not generate PCR products with identical ends: proofreading DNA polymerases leave blunt ends while blends of polymerases and non-proofreading DNA polymerases leaves 3´A overhangs. Therefore, it is necessary to employ different vectors to clone both kinds of PCR fragments.

pSpark® Universal DNA cloning kit has been designed to save time, looking for a kit for several cloning scenarios. It is mainly composed of two cloning vectors which allow blunt or TA DNA cloning. For blunt DNA cloning and TA DNA cloning, pSpark® II DNA Cloning vector and pSpark® TA DNA Cloning vector, respectively, are included.





3. Composition

Item	Quantity
pSpark® II DNA cloning vector	20 rxn (20ng/µL)
pSpark® TA DNA cloning vector	20 rxn (50ng/µL)
10X PEG 6000 solution	150 µL
1kb control insert	5 µL
600kb control insert (TA)	5 µL
T4 DNA ligase (5 Weiss U/µL)	20 µL
10x T4 DNA ligase Buffer	100 µL

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4. Features

- **Compatible with Blunt and TA DNA cloning:** it is composed by pSpark® II and pSpark® TA DNA Cloning vectors.
- **Convenient:** ideal for a broad range of PCR fragments cloning applications.
- **Versatile:** compatible with any DNA Polymerase.

5. Storage specifications

Store in a non-frost-free freezer at **-20°C.**

6. Applications

- Cloning of high-fidelity PCR amplified products into pSpark® II Blunt DNA cloning vector.
- Production of ssDNA.
- Cloning of non-proofreading PCR fragments into pSpark® TA DNA Cloning vector
- In vitro transcription from T7/SP6 dual-opposed promoters.

7. Recommended protocol

1. Amplification

Amplify DNA with any proofreading polymerase following suggested protocol. Gel purifies the insert or check by electrophoresis the quality of amplified DNA. If a single band appears on the gel and template DNA is not a vector with Ampicillin resistance, like the pSpark® II and TA DNA cloning vector, then gel purification is not needed.

2. Ligation

Suggested ratio of insert to vector is 5:1. Use the equation **ng insert = 33ng x kb** (of insert, that is, for a 1kb insert only 33 ng are needed) or use **1µL** of unpurified PCR product for directly ligation.

	Quantity	
pSpark® II DNA cloning vector	1 µL	-
pSpark® TA DNA cloning vector	-	1 µL
insert	X µL	X µL
10X PEG 6000 solution	1 µL	-
T4 DNA ligase (5 Weiss U/µL)	1 µL	1 µL
10x T4 DNA ligase Buffer	2 µL	2 µL
H_2O	Up to 10 µL	Up to 10 µL

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3. Transformation

Transformation can be made with your regular protocol, competent cells and selective media. As pSpark® II and TA DNA cloning vector has Ampicillin resistance, this antibiotic is needed. Also, as pSpark® II and TA DNA cloning vector has blue/white screening capability, IPTG/XGal plates are recommended.
A suitable transformation protocol is:

- Thaw competents cells on ice, take **50 µL** and put into a cool 1,5 mL tube.
- Add **10 µL of the ligation mixture**, mix by gently flicking with fingertips and incubate on ice for 30 minutes.
- Heat-shock the cells at **exactly 42°C for exactly 45 seconds** (do not shake) and then transfer to ice for 2 minutes.
- Plate the cells into media with antibiotic, IPTG and XGal and incubate overnight at 37°C.

4. Analysis of transformants

Either colony PCR or plasmid purification and digestion could be used for analysis of transformants. pSpark® II and TA DNA cloning vector has pUC/M13 forward and reverse sequencing primers binding site for sequencing or amplification of insert. Insert size when amplified with pUC/M13 fw and rev primers is about 200bp longer than the real size of insert.

5. Expected results

Up to 2500 positive white colonies for a 60 min ligation of 33 ng of supplied control insert (1 kb) and less than 20 negative blue colonies when using cells with a transformation efficiency of 4 x 10^7 cfu/ug..

7. Further information

Product Use Limitations This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.