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PRODUCT: Matchmaker™ Chemiluminescent Co-IP Vector Set

CATALOG No. 630458

LOT NUMBER

Specified on product label.

STORAGE CONDITIONS

Store all components at -20°C .

SHELF LIFE

1 year from date of receipt under proper storage conditions.

SHIPPING CONDITIONS

Dry ice (-70°C)

OTHER

- User Manual (PT3929-1)
- pAcGFP1-C Vector Information (PT3933-5)
- pProLabel-T Vector Information (PT3934-5)
- pProLabel-C Vector Information (PT3935-5)
- pAcGFP1-Lam Vector Information (PT3936-5)
- pAcGFP1-p53 Vector Information (PT3937-5)

DESCRIPTION

The Matchmaker Chemiluminescent Co-IP Vector Set contains two mammalian expression vectors, pAcGFP1-C and pProLabel-C. The set also contains two sets of predesigned and tested universal In-Fusion™ primers for simple and efficient In-Fusion PCR cloning of the bait and prey sequences from yeast two-hybrid expression constructs (pGBKT7-bait and pGADT7-prey) to generate N-terminal AcGFP-bait and ProLabel-prey fusions. These fusion constructs can be transfected into mammalian cells for confirmation of bait-prey protein interactions through coimmunoprecipitation studies using the Matchmaker Chemiluminescent Co-IP Assay Kit (Cat. No. 630459). The Matchmaker Chemiluminescent Co-IP Vector Set also provides a set of control vectors for conducting positive and negative control coimmunoprecipitations: pAcGFP1-Lam (negative bait), pAcGFP1-p53 (positive bait) and pProLabel-T (prey).

PACKAGE CONTENTS

- 10 μg pAcGFP1-C Vector (500 ng/ μl , circular)
- 10 μg pProLabel-C Vector (500 ng/ μl , circular)
- 10 μg pAcGFP1-Lam Vector (500 ng/ μl , circular)
- 10 μg pAcGFP1-p53 Vector (500 ng/ μl , circular)
- 10 μg pProLabel-T Vector (500 ng/ μl , circular)
- 40 μl AcGFP1 BD FWD Primer (10 μM)
- 40 μl AcGFP1 BD REV Primer (10 μM)
- 40 μl PL AD FWD Primer (10 μM)
- 40 μl PL AD REV Primer (10 μM)



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FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

See back of page.

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QUALITY CONTROL DATA

1. Plasmid identity and purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on an agarose/EtBr gel.

<u>Vector</u>	<u>Enzyme</u>	<u>Fragment Size (kb)</u>
pProLabel-C	Sal I or BamH I	4.2
	Pvu I/Mlu I	0.3 & 3.8
pProLabel-T	Pvu I/Mlu I	2.4 & 3.8
pAcGFP1-C	Sal I or Hind III	4.7
	Nhe I/Eag I	2.1 & 2.6
pAcGFP1-Lam	Nhe I/Sac I	0.9 & 4.6
pAcGFP1-p53	Nhe I/Kpn I	1.3 & 4.7

- The presence of the correct fluorescent protein variant was confirmed by sequencing.
- The purity of each plasmid was verified spectrophotometrically and the A_{260}/A_{280} ratio was found to be 1.8–2.0.

2. Primer testing

The AcGFP1 BD FWD/REV primer set was tested by amplification of the MCS region and the p53 sequence in pGBKT7-53 using Advantage® HD Polymerase Mix (Cat. No. 639241). After 30 amplification cycles, the PCR product was electrophoresed on a 1.0% agarose/EtBr gel. A major band of 1.2 kb was observed. The PL AD FWD/REV primer set was tested by amplification of the MCS region and the SV40 T sequence in pGADT7-T using Advantage HD Polymerase Mix. After 30 amplification cycles, the PCR product was electrophoresed on a 1.0% agarose/EtBr gel. A major band of 2.0 kb was observed.

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AcGFP is covered by U.S. Patent No. 7,432,053.

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