3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

# Product Information

**Director-Ready™ pT7-MAT™-2 SET**

Catalog Number **T6825**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Director-Ready vectors are pre-digested plasmid vectors that are ready for ligation to compatible inserts generated using traditional methods or by using the Director™ Universal PCR System, Catalog Number RDC1. Efficiencies for obtaining a positive recombinant clone are typically greater than 90% when used in conjunction with the Director Universal PCR System. The system also provides a simple, rapid and universal method to directionally clone and express PCR products.

Director-Ready pT7-MAT™-2 is a *Hin*d III/*Bgl* II digest of the 4.8 bp *Escherichia coli* expression vector pT7-MAT-2, Catalog Number E5655. Director-Ready pT7-MAT-2 may be used to clone any insert containing a 5' *Hin*d III compatible end and a 3' *Bgl* II compatible end.

pT7-MAT-2 is used for cytoplasmic expression of a properly inserted open reading frame as a C-terminal MAT™ (Metal Affinity Tag) fusion protein. The MAT tag (HNHRHKH) is a transition metal binding (e.g. $Ni^{+2}$, $Co^{+2}$, etc.) sequence useful for high quality purification. The promoter region of the very strong phage T7 promoter[1,2] drives transcription of ORF-MAT fusion constructs. This vector requires the use of *E. coli* cells containing a source of the T7 RNA polymerase, such as BL21(DE3) cells, Catalog Nos. B2935 or B3310. Transcription is regulated in these cells by having the T7 RNA polymerase gene under the control of the inducible *lacUV5* promoter. Tighter repression of basal level transcription is provided by the inclusion of *lacO* sequences immediately downstream of the T7 promoter and having the *lac* repressor gene (*lacI)* on the plasmid.

C-terminal MAT-tagged fusion proteins may be purified utilizing the metal affinity properties of the MAT tag by using HIS-Select® Nickel Affinity Gel, Catalog Number P6611. Sigma-Aldrich offers a wide selection of related HIS-Select products. Please visit www.sigma-aldrich.com for a complete listing of resins and affinity capture plates.

The following table provides map positions to key features in the Director-Ready pT7-MAT-2 vector. Sequence verification of the MCS can be performed using Verification primer-T7F and Verification Primer-T7R (included in the kit). Custom primers are available from Sigma-Genosys.

**Director-Ready pT7-MAT-2 Features**

| Feature | Map Position |
|---|---|
| *Bgl* II compatible end | 1-5 |
| MAT tag | 6-26 |
| T1/T2 terminator | 83-453 |
| Verification Primer-T7R | 161-182 |
| β-lactamase ($amp^r$) | 545-1405 |
| pBR322 ori | 1617-1736 |
| f1 ori | 2400-2863 |
| *lacI* | 3541-4623 |
| T7 Promoter | 4695-4714 |
| Verification Primer-T7F | 4654-4676 |
| Ribosomal Binding Site | 4766-4771 |
| *Hin*d III compatible end | 4782-4786 |

Refer to vector map

### Components/Reagents

- Director-Ready pT7-MAT-2, 1.5 µg, Catalog No. D3442
  50 ng/µl in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.
- Verification Primer-T7F (with 5' phosphorylation), Catalog No. V3889
  50 µl of 20 µM primer in, 10 mM Tris, pH 8.0, 1.0 mM. Sequence 5'-CTA TCA TGC CAT ACC GCG AAA GC-3'
- Verification Primer-T7R (with 5' phosphorylation, Catalog No. V3764
  50 µl of 20 µM primer in, 10 mM Tris, pH 8.0, 1.0 mM. Sequence 5'-CTA CGG CGT TTC ACT TCT GAG T-3'

**Additional Reagents and Equipment Not Supplied**

- DNA insert with 5' *Hin*d III compatible end and 3' *Bgl* II compatible end
- Dedicated pipets and aerosol resistant pipette tips
- Optional: Director Universal PCR System, Catalog No. RDC1
- T4 DNA Ligase and buffer
- LB-Ampicillin agar plates (100 µg/ml ampicillin)
- Molecular Biology Grade Water, Catalog No. W4502 or W1754
- Jumpstart™ REDTaq® ReadyMix™ DNA Polymerase (2x), Catalog No. P0982
- Thermal cycler

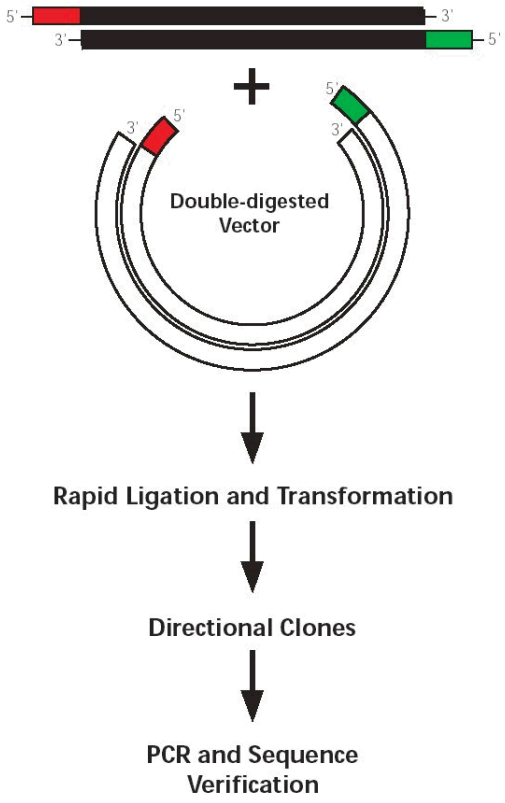
**Precautions and Disclaimer**

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

**Storage/Stability**

This product ships on dry ice and storage at –20 °C is recommended.

**Overview Diagram**




**Procedure**

## I. Ligation Reaction with Director Ready™ Vectors

Note: The insert used for the ligation reaction must contain a 5' *Hin*d III compatible end and a 3' *Bgl* II compatible end. The insert may be generated using traditional methods or through use of the Director Universal PCR System (Product No. RDC-1). Please refer to the Director system manual for instructions on generating the PCR fragment if this is the chosen method. Inserts should be purified and free of contaminants. Control inserts generated using RDC-1 are compatible with Director-Ready pT7-MAT-2.

1. Thaw Director-Ready pT7-MAT-2 vector. Vortex briefly and spin the tube for a few seconds. Once thawed, keep on ice.

2. Set up the ligation reaction as follows for both the control (RDC-1 kit) and for the insert of choice. Adjust volume of water for different amounts of insert.

| | |
|---|---|
| 8 µl | Water |
| 8 µl | Control insert or purified sample insert (approximately 250 ng) |
| 1 µl | Director Ready™ pT7-MAT-2 (50 ng) |
| 2 µl | 10x Ligation buffer |
| 19 µl total volume | |

3. Add 1µl of T4 DNA Ligase to each reaction. Mix thoroughly and incubate at room temperature for 60 minutes.

Note: Typically, 60 minutes is sufficient for ligations performed with optimal insert:vector ratios. A longer time may be required if reactions are performed with suboptimal ratios. Optimal insert:vector molar ratios for Director-Ready vectors is 5-10:1, which is higher than the conventional 3:1 ratio used for cohesive-end ligations. Extreme insert size could also alter the molar ratio for optimal ligation performance.

Calculate the molar ratio of insert to vector by the equation:

$$\text{Molar ratio of insert:vector} = (M_i/M_v) \times (S_v/S_i)$$

Where: $M_i$ = insert mass
$M_v$ = vector mass
$S_v$ = vector size
$S_i$ = insert size

4. Transform ligation reactions according to the competent cell manufacturer's instructions. Cloning strains with transformation efficiencies of ≥1 x $10^7$ cfu/μg DNA are recommended. Verified clones should be subsequently transformed into (DE3) derivatives such as BL21 (DE3) for protein expression.

**II. Verification of Transformants**

Note: A variety of methods can be used to verify positive transformants including restriction digests, sequencing and PCR. Since sequencing/verification primers are included, the following protocol is recommended for colony-based PCR.

1. Obtain overnight plates from transformation.

2. Pick colonies (~1mm in diameter) from freshly grown plate (using pipet tip or similar) and resuspend each in 50 μl Molecular Biology Reagent Water.

3. Use 9.2 μl for PCR amplification and keep the rest at 4 °C for additional culturing following verification.

4. PCR Set up

| | |
|---|---|
| 9.2 μl | Colony suspension |
| 10 μl | Jumpstart REDTaq ReadyMix DNA Polymerase (2x) |
| 0.4 μl | Verification Primer-T7R (20 μM) |
| 0.4 μl | Verification Primer-T7F (20 μM) |
| 20 μl Total Volume | |

5. PCR Profile

| | |
|---|---|
| Initial Denaturation | 94 °C, 5 minutes |
| | |
| 30 Cycles: | |
| Denaturation | 94 °C, 30 seconds |
| Annealing | 62 °C, 30 seconds |
| Annealing/Extension | 68 °C, 3 minutes |
| | |
| Soak | 4 °C |

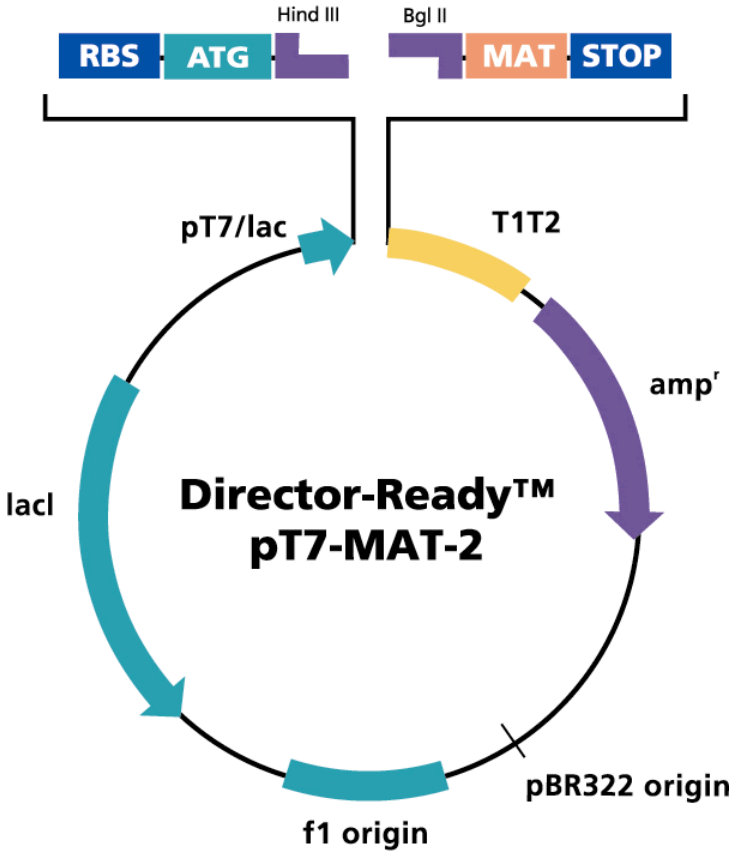
6. Analyze PCR amplicons by gel electrophoresis. Five μl of PCR reactions may be loaded directly onto the gel for analysis.

7. Positive clones will be 289 bp plus the size of the target insert.

**References**

1. Studier, F. W., and Moffatt, B. A., Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol., **189,** 113-130 (1986).
2. Rosenberg, A. H., et al., Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene, **56**, 125-135 (1987).

**Director-Ready pT7-MAT-2 (4.8 kb)**




## Cloning Site

(Director-Ready pT7-MAT-2)