

Directional RH cDNA Library Construction Systems

The Directional RH cDNA Library Construction Systems are complete sets of reagents designed for rapid, efficient construction of cDNA libraries having inserts in a defined orientation. Two systems are available that differ only in the primer used for first strand synthesis.

The **Directional RH cDNA Library Construction Systems** include the following kit modules for construction of up to 5 libraries:

- Directional RH Random Primer cDNA Synthesis Kit or Oligo(dT) Primer cDNA Synthesis Kit
- *EcoR I/Hind III* End Modification Kit
- DNA Ligation Kit
- Mini Column Fractionation Kit
- λ SCREEN-1 *EcoR I/Hind III* Arms plus PhageMaker Kit
- Protocol

Product	Cat. #	Price
Directional RH Random Primer cDNA Library Construction System	69990-1	
Directional RH Oligo(dT) cDNA Library Construction System	69991-1	

Kits available separately:

Product	Cat. #	Price
Directional RH Random Primer cDNA Synthesis Kit	69992-1	
Oligo(dT) Primer cDNA Synthesis Kit	69993-1	
<i>EcoR I/Hind III</i> End Modification Kit	69994-1	
DNA Ligation Kit	69838-1	
Mini Column Fractionation Kit	69995-1	
λ SCREEN-1 <i>EcoR I/Hind III</i> Arms Kit plus PhageMaker	69985-1	
Straight A's™ mRNA Isolation System + Separation Stand	69962-1	

Stock cDNA Libraries

Novagen offers two new cDNA libraries constructed in our advanced autosubcloning vector, λ SCREEN-1, using directional random primed methodology. Each library contains $> 10^6$ primary recombinants and has undergone a single round of amplification. cDNAs were size fractionated such that inserts are > 300 bp in length. The size range of inserts has been verified to be from 300bp to > 2000 bp by gel electrophoresis of DNA prepared from the amplified libraries. The libraries have been constructed without the use of carrier RNA, which eliminates the

possibility of contamination with heterologous clones.

Each Stock Library Kit contains the following components:

- 100 μ l aliquot of phage at $>10^9$ pfu/ml (enough for 50–500 screenings)
- Host strains
- Protocol

Product	Cat. #	Price
K562 cell (uninduced) cDNA Library	69011-1	
Friend cell (uninduced) cDNA Library	69012-1	

Thrombin Cleavage Capture Kit

Restriction Grade Biotinylated Thrombin is qualified to specifically cleave target proteins produced with appropriate vectors. It has been covalently attached to biotin for easy removal of the enzyme from cleavage reactions using immobilized streptavidin. The preparation is functionally tested for activity with fusion proteins and is free of detectable contaminating proteases. It is also certified for $>99\%$ binding to Streptavidin Agarose.

Each **Thrombin Cleavage Capture Kit** contains the following components:

- 50units Biotinylated Thrombin
- 3ml 10X Thrombin Cleavage Buffer
- 0.4ml Streptavidin Agarose
- Protocol

Product	Cat. #	Price
Thrombin Cleavage Capture Kit	69022-1	

pET TRX Fusion System 32

The pET TRX Fusion System 32 is designed for cloning and high-level expression of peptide sequences fused with the 109aa Trx•Tag thioredoxin protein (see article beginning on p. 7). The system has several features that facilitate the expression and purification of soluble target proteins. Cloning sites are available for producing fusion proteins also containing cleavable His•Tag and S•Tag sequences for detection and purification.

The **pET TRX Fusion System 32** is available as a choice of kits with individual components available separately. The basic system contains:

- 10µg each pET-32a(+), 32b(+) and 32c(+) DNA
- Host bacterial strains AD494(DE3), AD494(DE3)pLysS, BL21, BL21(DE3) and BL21(DE3)pLysS
- 10ml His•Bind Resin
- 4 polypropylene chromatography columns with closures
- Vector map and protocols
- Novagen Vector Diskette containing all Novagen plasmid sequences (specify Macintosh or DOS format when ordering)

The **System plus Competent Cells** contains all components listed above plus host strains ready for high efficiency transforma-

tion. One 0.2ml aliquot each of the initial cloning host NovaBlue and the expression hosts AD494(DE3), AD494(DE3)pLysS, BL21(DE3) and BL21(DE3)pLysS plus SOC medium are included.

Product	Size	Cat. #	Price
pET TRX Fusion System 32		69018-1	
pET TRX Fusion System 32 plus Competent Cells		69019-1	
pET-32a(+) DNA	10µg	69015-1	
pET-32b(+) DNA	10µg	69016-1	
pET-32c(+) DNA	10µg	69017-1	

AD494(DE3), AD494(DE3)pLysS

Strains AD494(DE3) and AD494(DE3)pLysS are derived from the thioredoxin reductase (*trxB*) mutant AD494, which allows the formation of disulfide bonds in the cytoplasm (1; see article on p. 9). These strains are λDE3 lysogens, which contain a chromosomal copy of the T7 RNA polymerase gene under *lacUV5* control. The pLysS version also carries a compatible chloramphenicol resistant plasmid that encodes T7 lysozyme, which provides more stringent control of basal expression levels. The *trxB*

mutation is selectable on kanamycin; therefore, this strain is recommended for use with pET plasmids carrying the Ap resistance marker *bla*.

Both strains are available as pretested competent cells ready for transformation and as glycerol stocks. SOC medium and test plasmid are included with competent cells.

1. Derman, A.I., Prinz, W.A., Belin, D. and Beckwith, J. (1993) *Science* **262**, 1744-1747.

Product	Size	Cat. #	Price
AD494(DE3) Comp. Cells	0.4ml 1ml	69013-1 69013-2	
AD494(DE3)pLysS Comp Cells	0.4ml 1ml	69014-1 69014-2	
AD494(DE3) Glycerol Stock	0.2ml	69020-1	
AD494(DE3)pLysS Glycerol Stock	0.2ml	69021-1	

M13mp18 Blunt-End Cloning Kit

M13mp18 is a popular single-stranded filamentous bacteriophage cloning vector designed for easy preparation of high-quality sequencing templates (1).

The M13mp18 Blunt-End Cloning Kit is specifically qualified for blunt-end cloning of DNA fragments for sequence analysis. The *Sma* I digested, dephosphorylated M13mp18 DNA is rigorously tested for optimal cloning efficiency with minimal background; typically >90% clear plaques are obtained after ligation of blunt-ended fragments at a 10-fold molar excess with Novagen's ligation reagents and NovaBlue Competent Cells. This product is suitable for direct cloning of any type of blunt-ended DNA up to 1000bp in size.

A T-Vector version of M13mp18 is also available for cloning fragments having single 3' dA overhangs.

1. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene* **33**, 103-119.

Each **M13mp18 Blunt-End Cloning Kit** contains the following components (40 ligations and transformations):

- 2µg *Sma* I digested dephosphorylated M13mp18 DNA
- Blunt-ended Positive Control Insert
- 200µl NovaBlue Glycerol Stock
- 5 × 200µl NovaBlue Competent Cells
- 4 × 2ml SOC medium
- Test Plasmid for Transformation
- Protocol

Product	Size	Cat. #	Price
M13mp18 Blunt-End Cloning Kit		69996-1	
<i>Sma</i> I digested dephosphorylated M13mp18 DNA	2µg	69981-1	
M13mp18 RF I DNA	10µg	69980-1	
NovaBlue Competent Cells	0.4ml 1ml	69825-1 69825-2	
Introductory M13mp18 T-Vector Kit		69979-1	
Regular M13mp18 T-Vector Kit		69977-1	
Regular M13mp18 T-Vector Kit plus Ligase		69978-1	
M13mp18 T-Vector	2µg	69976-1	

T7•Tag Antibody Alkaline Phosphatase Conjugate

The T7•Tag monoclonal antibody recognizes the amino terminal 11aa of the T7 gene 10 protein expressed from many pET vectors, as well as from pTOPE®, pSCREEN, λEXlox, pRSET, and pGEMEX vectors. It, therefore, serves as a highly specific reagent for detection of recombinant fusion proteins on blots, and by immunoprecipitation and immunofluorescence (1, 2). For increased versatility and sensitivity, we now offer this antibody conjugated with alkaline phosphatase. This form of the antibody is useful as a single detection reagent for Western blots which eliminates the need for second antibodies or streptavidin conju-

gates. It produces very clean blots since there is no possibility of detecting non-target molecules that may react with second antibody or streptavidin conjugates. The 50µl size provides enough conjugate for 50 Western blots.

1. Lutz-Freyermuth, C., Query, C.C. and Keene, J.D. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6393-6397.
2. Tsai, D.E., Kenan, D.J. and Keene, J.D. (1992) *Proc. Natl. Acad. Sci. USA* **88**, 8864-8868.

Product	Size	Cat. #	Price
T7•Tag Antibody AP Conj.	50µl	69999-1	

T7•Tag Affinity Purification Kit

The T7•Tag Affinity Purification Kit is designed for rapid immunoaffinity purification of target proteins that carry the 11aa T7•Tag sequence (*i.e.*, the initial 11aa of the T7 gene 10 protein). Purification is based on binding target proteins to T7•Tag monoclonal antibody which is covalently coupled to cross-linked agarose beads, washing away unbound proteins, and eluting at pH 2.2. Capacity will vary somewhat between different target proteins, but the beads are standardized to bind a minimum of 300µg T7•Tag β-galactosidase per ml of settled resin. The beads can be used in

either batch or column modes and can be recycled a minimum of five times without loss of binding activity. The kit includes the following reagents:

- 1ml T7•Tag Antibody Agarose
- 20ml 10X Bind/Wash Buffer
- 20ml 10X Elute Buffer
- 20ml Neutralization Buffer
- chromatography column
- Protocol

Product	Size	Cat. #	Price
T7•Tag Affinity Purif. Kit		69025-1	
T7•Tag Antibody Agarose	2ml	69026-1	

