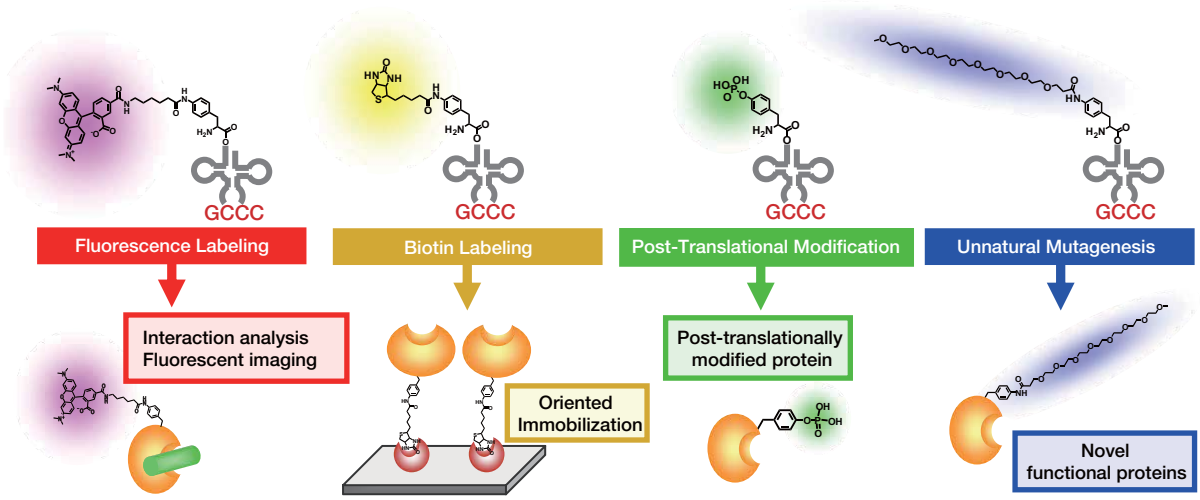
CloverDirect™

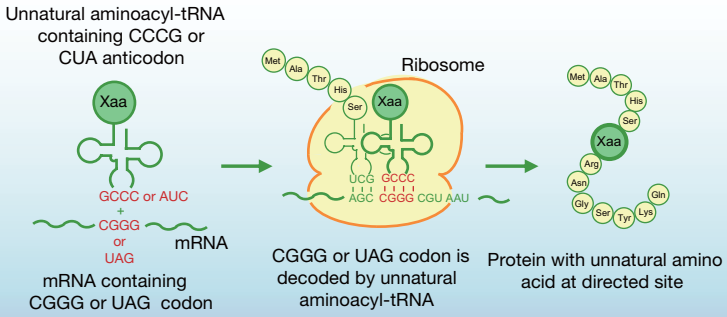
tRNA Reagents for Site-Directed Protein Functionalization

Expression of proteins with unnatural amino acids using four-base codon (CGGG) or amber stop codon (UAG)

New Tool For Protein Research

Clover*Direct*™ tRNA Reagents for Site-Directed Protein Functionalization allow the incorporation of unnatural amino acids at defined positions of proteins using in vitro translation system. Various unnatural amino acids are available for fluorescence labeling, biotin labeling, post-translational modification, and unnatural mutagenesis of proteins.





Principle

If CGGG or UAG codon is recognized by unnatural aminoacyl-tRNA, full-length protein with unnatural amino acid is successfully synthesized. On the contrary, if CGGG or UAG codon is recognized by natural translation factor (arginyl-$tRNA_{CCG}$ or release factor 1), the protein synthesis is terminated. Therefore, the translation product obtained as a full-length protein contains the unnatural amino acid at 100% efficiency.

Features

Accurate and Quantitative:

Incorporation position of unnatural amino acids is defined by CGGG four-base codon or UAG amber codon. An unnatural aminoacyl-tRNA recognizes the CGGG or UAG codon during translation to introduce unnatural amino acids into proteins in a site-directed and quantitative fashion.

Fast and Easy:

Proteins with unnatural amino acids can be obtained within a few hours just by adding Clover*Direct*™ reagents and DNA or mRNA having CGGG or UAG codon to an ***in vitro*** translation system.

Flexible:

Various unnatural amino acids containing fluorescent groups, biotin, PEG, photo-cross-linker, *etc.* are available.

Product Contents

· Unnatural aminoacyl-tRNA X 1
· tRNA dissolving buffer X 1

One tube contains unnatural aminoacyl-tRNA sufficient for 300 µL of ***in vitro*** translation reaction.

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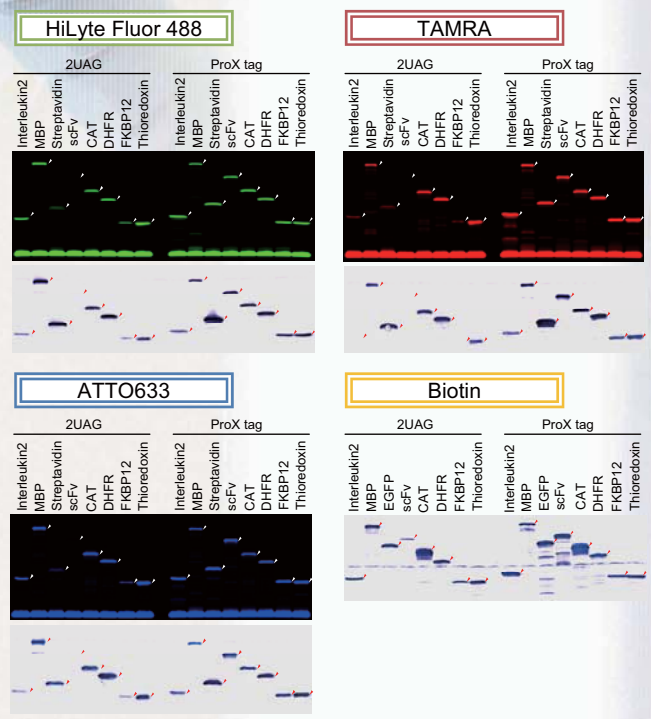
Applications of site-directly labeled proteins

Site-directly fluorescent-labeled proteins are available to the following analyses.

- Protein interaction analysis using Single Molecule fluorescence detection system (MF20 / FluoroPoint-Light; OLYMPUS).
- Conformation analysis of protein by inter- or intra-molecular fluorescence resonance energy transfer (FRET).
- Functional analysis by cell imaging.
- Interaction analysis by protein array.
- Expression analysis by fluorescent detection of SDS-PAGE.

Expression of site-directly labeled proteins

Fluorescent- and biotin-labeled unnatural amino acids are incorporated into eight prokaryote and eukaryote proteins. The site-directly fluorescent-labeled proteins can be visualized on SDS-PAGE using a laser-based fluorescence scanner. The proteins are also detectable by an antibody against tag peptide or biotin.



2UAG : UAG codon is inserted after initiator AUG codon.
ProX tag : ProX tag is fused to the N-terminus.
Applied volume: 0.25 µL of translational reaction mix
Fluorescence images (Top) are visualized with Ex and Em wavelengths listed below:

HiLyte Fluor488	Ex : 488 nm / Em : 520 nm
TAMRA	Ex : 532 nm / Em : 580 nm
ATTO633	Ex : 635 nm / Em : 670 nm

Western blotting (Bottom) are analyzed by anti-His tag antibody (for fluorescent amino acids) and anti-biotin antibody (for biotin)

References

1) Daisuke Kajihara, Ryoji Abe, Issei Iijima, Chie Komiyama, Masahiko Sisido, Takahiro Hohsaka *Nature Methods*, **3**, 923-929 (2006).
2) Takayoshi Watanabe, Norihito Muranaka, Issei Iijima, Takahiro Hohsaka *Biochem. Biophys. Res. Commun.*, **361**, 794-799 (2007).
3) Hikaru Taira, Yosuke Matsushita, Kenji Kojima, Kaori Shiraga, Takahiro Hohsaka Biochem. *Biophys. Res. Commun.*, **374**, 304-308 (2008).
4) Takahiro Hohsaka, Daisuke Kajihara, Yuki Ashizuka, Hiroshi Murakami, Masahiko Sisido *J. Am. Chem. Soc.*, **121**, 34-40 (1999).

Product List

Site-Directed Fluorescence Labeling

Catalog No.	Description	Size
PRX-CLD1001	CloverDirect™ CR110-X-AF (amber)	300 µL
PRX-CLD2001	CloverDirect™ CR110-X-AF (amber)	5×300 µL
PRX-CLD2002	CloverDirect™ CR110-X-AF (CGGG)	5×300 µL
PRX-CLD01	CloverDirect™ HiLyte Fluor 488-AF (amber)	300 µL
PRX-CLD05	CloverDirect™ HiLyte Fluor 488-AF (amber)	5×300 µL
PRX-CLD2004	CloverDirect™ HiLyte Fluor 488-AF (CGGG)	5×300 µL
PRX-CLD02	CloverDirect™ TAMRA-X-AF (amber)	300 µL
PRX-CLD06	CloverDirect™ TAMRA-X-AF (amber)	5×300 µL
PRX-CLD03	CloverDirect™ ATTO633-AF (amber)	300 µL
PRX-CLD07	CloverDirect™ ATTO633-AF (amber)	5×300 µL
PRX-CLD2008	CloverDirect™ ATTO633-AF (CGGG)	5×300 µL
PRX-CLD1009	CloverDirect™ ATTO655-X-AF (amber)	300 µL
PRX-CLD2009	CloverDirect™ ATTO655-X-AF (amber)	5×300 µL
PRX-CLD1010	CloverDirect™ ATTO655-X-AF (CGGG)	300 µL
PRX-CLD2010	CloverDirect™ ATTO655-X-AF (CGGG)	5×300 µL

Site-Directed Biotin Labeling

Catalog No.	Description	Size
PRX-CLD2101	CloverDirect™ Biotin-AF (amber)	5×300 µL
PRX-CLD2102	CloverDirect™ Biotin-AF (amber)	5×300 µL
PRX-CLD2103	CloverDirect™ Biotin-X-AF (amber)	5×300 µL
PRX-CLD2104	CloverDirect™ Biotin-X-AF (CGGG)	5×300 µL
PRX-CLD04	CloverDirect™ Biotin-XX-AF (amber)	300 µL
PRX-CLD08	CloverDirect™ Biotin-XX-AF (amber)	5×300 µL
PRX-CLD2106	CloverDirect™ Biotin-XX-AF (CGGG)	5×300 µL

Site-Directed Post-Translational Modification

Catalog No.	Description	Size
PRX-CLD2201	CloverDirect™ Tyr(PO3H2) (amber)	5×300 µL
PRX-CLD2202	CloverDirect™ Tyr(PO3H2) (CGGG)	5×300 µL
PRX-CLD2203	CloverDirect™ Lys(Me) (amber)	5×300 µL
PRX-CLD2204	CloverDirect™ Lys(Me) (CGGG)	5×300 µL
PRX-CLD2205	CloverDirect™ Lys(Me2) (amber)	5×300 µL
PRX-CLD2206	CloverDirect™ Lys(Me2) (CGGG)	5×300 µL
PRX-CLD2207	CloverDirect™ Lys(Ac) (amber)	5×300 µL
PRX-CLD2208	CloverDirect™ Lys(Ac) (CGGG)	5×300 µL

Site-Directed Unnatural Mutagenesis

Catalog No.	Description	Size
PRX-CLD2301	CloverDirect™ PEG4-AF (amber)	5×300 µL
PRX-CLD2302	CloverDirect™ PEG4-AF (CGGG)	5×300 µL
PRX-CLD2303	CloverDirect™ PEG8-AF (amber)	5×300 µL
PRX-CLD2304	CloverDirect™ PEG8-AF (CGGG)	5×300 µL
PRX-CLD2305	CloverDirect™ PEG12-AF (amber)	5×300 µL
PRX-CLD2306	CloverDirect™ PEG12-AF (CGGG)	5×300 µL
PRX-CLD2321	CloverDirect™ BPA (amber)	5×300 µL
PRX-CLD2322	CloverDirect™ BPA (CGGG)	5×300 µL
PRX-CLD2323	CloverDirect™ AcPhe (amber)	5×300 µL
PRX-CLD2324	CloverDirect™ AcPhe (CGGG)	5×300 µL
PRX-CLD2331	CloverDirect™ AzoAla (amber)	5×300 µL
PRX-CLD2332	CloverDirect™ AzoAla (CGGG)	5×300 µL

We provide custom services for the synthesis of unnatural aminoacyl-tRNAs and the expression of proteins with unnatural amino acids.

manufacturer: ProteinExpress

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