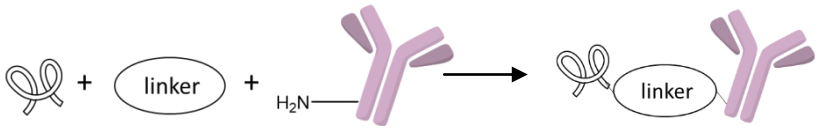
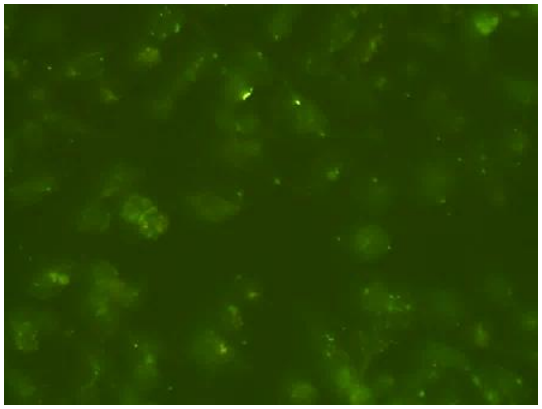
ICDS™ kit

Introduction

ICDS™ peptides can deliver biologically active molecules across cellular membranes into cells, which was an important breakthrough in cell biology. This ICDS™ kit provides a convenient method to conjugate ICDS™ peptides with proteins, and then deliver the proteins into cells with ICDS™ peptides as a carrier. This kit can be used with a wide range of protein concentrations and with virtually any protein larger than 20 kDa. The linker provided in this kit conjugates with primary amine group of proteins first, and then the ICDS™ peptides are added to form the ICDS™ peptides -protein conjugates.





The ICDS™ peptides-protein conjugates transfer into cells

Kit Contents

ICDS™ peptides: 1 mg.
Linker: 2 mg.
Buffer: PBS solution (5 mL).
Filtration Tube: 2 tubes.

Storage Condition

-20 °C

Required Equipment

Centrifuge.
Tubes.

Calculations

Perform the following calculation before beginning the conjugation reaction.

The amount of linker and ICDS™ peptides to be used for each reaction depends upon the concentration of protein to be labeled.

$$\mu\text{L reactive linker stock solution} = \frac{\text{mg protein}}{\text{MW}_{\text{protein}}} \times R_l \times 220{,}000$$

- R_l is the molar ratio of linker to protein in the reaction mixture. We suggest that R_l= 40 if the protein is at 1–10 mg/mL.
- For most whole IgGs, $\text{MW}_{\text{protein}}$ = 145,000.
- 220,000 is a unit conversion factor.

$$\mu\text{L reactive ICDSTM peptides stock solution} = \frac{\text{mg protein}}{\text{MW}_{\text{protein}}} \times R_c \times 410{,}000$$

- R_c is the molar ratio of ICDS™ peptides to protein in the reaction mixture. We suggest that R_c= 4 if the protein is at 1–10 mg/mL.
- For most whole IgGs, $\text{MW}_{\text{protein}}$ = 145,000.
- 410,000 is a unit conversion factor.

Preparing the Protein

Purified protein should be at a concentration of 1-10 mg/mL in buffer.

The buffer should not contain any ammonium ions or primary amines. If the protein in an unsuitable buffer (e.g., Tris or glycine), the buffer must be replaced by dialysis against PBS or by using the provided filtration tube. The buffer components permissible are in the table below.

Buffer components	
pH	6.5-8.5
Amine free buffer *(e.g. MES, MOPS, HEPES, PBS)*	✓
Primary amines *(e.g. Tris, glycine, ethanolamine)*	×
Non-buffering salts *(e.g. sodium chloride)*	✓
Chelating agents *(e.g. EDTA, EGTA)*	✓
sugars	✓
Glycerol	✓
Thiols *(e.g.mercaptoethanol,DTT)*	×
Preservative*(e.g. thimerosal, sodium azide)*	×
BSA	×

General Protocol

(1) Prepare the 2 mg/mL reactive linker stock solution: Add 1.0 mL water into the linker and make it dissolved.[(a)]

(2) Prepare the 5 mg/mL reactive ICDS™ peptides stock solution: Add 200 μL PBS buffer into the ICDS™ peptides and make it dissolved.

(3) Add the appropriate amount (see **Calculations**) linker stock solution to the protein. Incubate the tube at 25 °C for 30 min.

(4) Add the protein-linker conjugates solution in one filtration tube, and then centrifuge at 10,000 ×*g* for 10 min.[(b)]

(5) Add 300 μL PBS buffer to the filtration tube and centrifuge at 10,000 ×*g* for 10 min again.

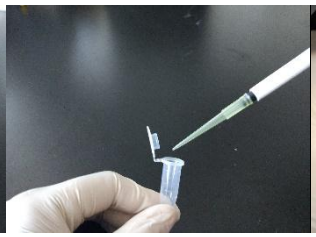
(6) Transfer the purified protein-linker conjugate solution to a microtube, and add the appropriate amount (see **Calculations**) ICDS™ peptides stock solution to it. React for 12 h at 4 °C or 2 h at room temperature.

(7) Add the ICDS™ peptides-protein conjugates solution in a new filtration tube, and then centrifuge at 10,000 ×*g* for 10 min. [(c)]

(8) Add 300 μL PBS buffer to the filtration tube and centrifuge at 10,000 ×*g* for 10 min again.

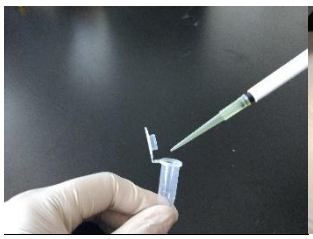
(9) Transfer the purified ICDS™ peptides-protein conjugates to a microtube, and it can be stored at 4 °C.

(1), (2) Prepare the stock solutions.



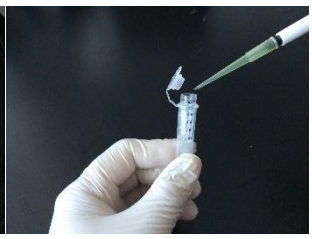
(3) Conjugation of the linker and protein.

(4), (5) Centrifuge to remove excess linkers.



(6) Conjugation of ICDS™ peptides.

(7), (8) Centrifuge to remove excess ICDS™ peptides.

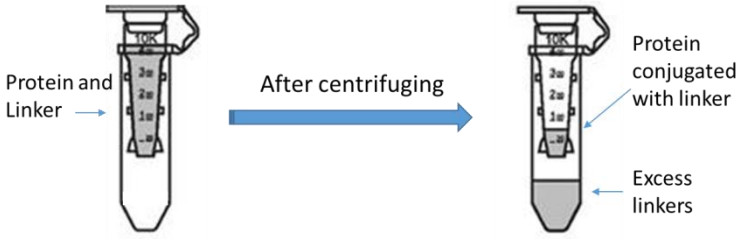


(9) Transfer the solution to a tube, and store at 4 °C.

Comments

(a) Do not use phosphate-buffered saline (PBS) for initial dissolution of the linker; the reagent does not dissolve well in buffers exceeding 50mM total salts. However, once dissolved, the solution can be further diluted in PBS or other non-amine buffers.

(b) Centrifuge to remove excess linkers:



(c) Centrifuge to remove excess ICDS™ peptides: