IMRA America, Inc.
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i-colloid™ Gold Nanoparticle Peptide Conjugation Kit

40 nm Gold Nanoparticles

INSTRUCTIONS FOR USE

This product is for **Research Use Only**

Overview

IMRA's i-colloid™ Gold Nanoparticle Peptide Conjugation Kit provides an expedient conjugation of peptide with gold nanoparticles. The method takes advantage of the high purity of IMRA's laser-made gold nanoparticles to enable rapid binding of cysteine-terminated peptide to the chemically-bare gold surface via high affinity gold-thiol bonds. The colloidal stability of the conjugates is ensured by pre-grafting polyethylene glycol (PEG) on the gold surface with precisely controlled partial coverage, leaving ample space for peptide conjugation without affecting peptide performance. This conjugation kit can be used for a wide range of applications such as cell staining and imaging and pharmaceutical peptide screening. This instruction details the procedure of conjugation of peptides with i-colloid™ 40 nm gold nanoparticles. Final yield is 1 ml conjugates of optical density (OD) 10 (0.5 mg/ml).

Product description

Catalog No. icAu40PC10 (10 Reactions)

Items included

Item	Material	Description
No. 1	Storage buffer	1x concentration, 4 ml
No. 2	Wash buffer	10x concentration, 4 ml
No. 3	Modified gold	40 nm PEG-gold nanoparticles, 1 ml, OD 10 (0.5 mg/ml)
No. 4	Negative control gold	40 nm bare gold nanoparticles, 0.5 ml, OD 10 (0.5 mg/ml)
No. 5 and 6	Conjugation reaction vials	Fisher Scientific (cat. no. 03-375-16AS)

Safety precautions

Adhere to standard lab safety procedures when handling chemical reagents.

Product compatibility

Modified gold nanoparticles are stable in the provided solutions. High ionic strength (e.g., >0.25 M NaCl) reagents will destabilize colloidal stability and induce aggregation. It is recommended to use the provided storage buffer (item No. 1) and wash buffer (item No. 2) and avoid high ionic strength as much as practically possible.

Peptide conjugation procedure

The following conjugation procedure applies to most cysteine-terminated peptides. Protocols can vary depending on applications.

Additional materials and equipment required

- Peptide of interest (modified with cysteine at the terminus distal to the binding site)

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- Deionized (DI) water (18.0 MΩ-cm)
- 1.5 ml centrifuge tubes and centrifuge machine
- Standard laboratory equipment such as pipettes and vortex mixers

Allow all reagents to come to room temperature before starting.

I. Modified gold preparation

1. Re-suspend the modified gold (item No. 3) by vortexing for 10 seconds on high speed.
2. Use pipette to transfer 100 µl of the modified gold to one of the conjugation reaction vials (item No. 5 or 6). The conjugation reaction vial can be reused later after rinsing with deionized water three times.

II. Peptide Conjugation

1. Dissolve appropriate amount of peptide of interest in DI water to prepare 100 µl or more peptide solution of 10 µM in the unused conjugation reaction vial (item No. 5 or 6). Peptide concentration can vary depending on peptide. The recommended concentration range is 7.5 - 12.5 µM for 40 nm modified gold of OD 10 at 1:1 volume ratio. Ensure that this solution is freshly prepared.
2. Use a pipette to transfer 100 µl peptide solution to the 100 µl modified gold suspension and mix by pipetting up and down, bringing the solution OD to 5.
3. Incubate on the bench for 30 minutes at room temperature.

III. Purification and storage

1. Use a pipette to transfer 100 µl of wash buffer (item No. 2) to a new 1.5 ml centrifuge tube. Dilute by 10 times by adding 900 µl DI water.
2. Dilute 200 µl of peptide gold conjugates made in step II from OD 5 to OD 1 by adding 800 µl diluted wash buffer prepared in step III.1. Mix by pipetting up and down. Transfer the entire volume (1 ml) to a new 1.5 ml centrifuge tube.
3. Centrifuge for 10 minutes at 1500 g at room temperature. Discard supernatant, which may appear slightly pink. Re-suspend the pellet, which should be dense but still fluidic, using storage buffer (item No. 1) back to OD 1 or your desired concentration using the volume recommendations below.

Desired OD	Volume of storage buffer to add (µL)
1	1000
2	500
4	250
10	100
15	67
20	50

4. The final solution now consists of gold nanoparticles functionalized with your peptide of interest and is ready to use.
5. It is recommended to run two identical tests, one with gold nanoparticles functionalized with peptides and the other with the negative control gold (item No. 4), and compare results for verifying the success of conjugation. The verification testing depends on your application, for example binding to specific target receptors.
6. Store the peptide conjugates at 4 °C. Do not expose to heat. Do not freeze.

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Release Information

Success of peptide conjugation described in this instruction is assessed by conjugation with RGD peptide with the sequence of RGDRGDRGDRGDPGC (from N-terminus to C-terminus). Specific targeted binding of RGD-gold conjugates to cancer cells overexpressing integrin receptors is confirmed with human HeLa cancer cells and compared with negative control.

Shipping and Storage

This product is shipped at ambient conditions. Store at 2 ^{o}C – 8 ^{o}C upon receiving the product. Remaining materials after use should be retained in the supplied container and sealed for future use. Do not expose to temperatures above 60 ^{o}C. Do not freeze.

Technical Support

For questions regarding this product and technical support, please visit our website http://nano.imra.com or contact us via telephone or email.