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# i-colloid™ Gold Nanoparticle Cell Imaging Kit

40 nm Gold Nanoparticles

## INSTRUCTIONS FOR USE

This product is for **Research Use Only**

### Overview

IMRA's i-colloid™ Gold Nanoparticle Cell Imaging Kit is designed for cell imaging with dark-field optical microscopy. The gold nanoparticles are conjugated with RGD peptide for cell targeting. Under dark field illumination, gold nanoparticles are $10^5$ to $10^6$ times brighter than organic dyes due to the particles' large optical scattering cross-section at the plasmon resonance wavelength. A low concentration of gold conjugates on the order of nM is sufficient to produce a sharp image. Gold nanoparticles are also resistant to photo-blinking and photo-bleaching, allowing continuous and extended cell imaging, tracking, and analysis. This instruction details the procedure for cell staining and dark-field optical microscopy imaging in cell culture media using this kit.

### Product Description

Catalog No.: icAu40CI10 (10 Reactions)

#### Items included (all sterilized)

| Item | Material | Description |
|---|---|---|
| No. 1 | Dilution Buffer | 10x concentrated, 2 ml |
| No. 2 | Dilution container | Micro tube, 2 ml, Polypropylene (Sarstedt, cat. no. 72.694.106) |
| No. 3 | Gold conjugates | 40 nm gold nanoparticle RGD peptide conjugates, 1 ml, OD 10 (0.5 mg/ml) |
| No. 4 | Negative control | 40 nm gold nanoparticles, 0.5 ml, OD 10 (0.5 mg/ml) |

### Safety Precautions

Adhere to standard lab safety procedures when handling chemical reagents.

### Product Compatibility

The gold conjugates are stable in the provided solutions. High ionic strength (e.g., >0.25 M NaCl) reagents will destabilize the colloid and induce aggregation. Dilution can be made by the dilution buffer (item No. 1) or cell culture media during cell staining (see step II below).

### Additional Materials and Equipment Needed

- Cancer cells of interest (e.g., human HeLa cells) cultured aseptically at 37 °C and 5% $CO_2$ in a humidified incubator
- Imaging dish: 35 mm glass bottom cell culture dishes (MatTek Corporation, Part No. P35G-0-14-C)
- Cell culture media: Dulbecco's modified Eagle medium (DMEM, Thermo Fisher Scientific, cat. no. 11995-065) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin.
- Cell rinse buffer: Dulbecco's Phosphate Buffered Saline (DPBS, Thermo Fisher Scientific, cat. no. 14190-144)

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- Optical microscope: with dark-field illumination. An inverted scope is preferred.
- Standard biological laboratory and cell culture equipment such as pipettes, class II biological safety cabinet, and $CO_2$ incubator for growing and maintaining cell cultures.

## Cell Staining and Imaging Procedure

The procedure below is based on staining and imaging human HeLa cancer cells. Protocols should be modified for individual applications.

### I. Cell preparation

1. Transfer a 1 ml suspension of cells of interest at a density between 1 x $10^4$ cells/ml and 2 x $10^4$ cells/ml prepared in cell culture medium into a new imaging dish and culture for additional 24 hours at 37 °C and 5% $CO_2$ in a humidified incubator. This allows the cells to attach to the surface of the imaging dish prior to initiating staining with the provided 40 nm gold conjugates.

### II. Gold conjugate dilution

1. Bring all components of the cell imaging kit and cell culture reagents into the biological safety cabinet. Allow the items and reagents to come to room temperature.
2. Use a pipette to mix the gold conjugates (item No. 3) by pipetting up and down, and then transfer 100 µl of the conjugate solution to the dilution container (item No. 2). The dilution container can be reused later after rinsing with cell rinse buffer for three times.
3. Dilute 100 µl of the gold nanoparticle conjugate solution from OD 10 (0.5 mg/ml) to OD 1 (0.05 mg/ml) by adding 900 µl cell culture media and mixing by pipetting up and down.

### III. Cell staining

1. Aspirate the original cell culture media from the imaging dish. Wash cells twice with 1 ml cell rinse buffer.
2. Add 1 ml gold conjugate solution of OD 1 (0.05 mg/ml) as prepared in step II.3 to the cell imaging dish. Incubate for 1 hour at 37 °C and 5% $CO_2$ in a humidified incubator for the cells to be stained with gold nanoparticles.
3. At the end of incubation, gently aspirate the media from the imaging dish. Wash with 1 ml cell rinse buffer three times to remove free gold conjugates. Leave the cells in the rinse buffer. The cells are now ready for optical microscopy imaging.

### IV. Imaging

1. An inverted optical microscope is preferred to image from below the dish. An objective lens of up to 50x may be used and is contingent upon working distance. Under dark-field illumination, cells stained with gold nanoparticles mostly appear green, which is attributable to enhanced light scattering by the gold nanoparticles. Yellow and red colors occasionally appear at high concentration.
2. It is recommend to run a negative control test to confirm the specification of cell staining by replacing the gold conjugates with negative control (item No. 4) in steps II and III.

### V. Cell fixation (OPTIONAL)

Cell fixation is suggested if long-term cell imaging and analysis is required.

After cell staining, the cells can be fixed onto the imaging dish by adding 0.1 ml fresh 4% paraformaldehyde in PBS and incubating at ambient conditions for 15 minutes before

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washing three times with 1 ml cell rinse buffer. Leave the cells in the cell rinse buffer. The cells are now ready for optical microscopy. Store the fixed cells at 4°C when they are not being used. Do not freeze.

**Release Information**

The functionality of specific cell staining of the gold conjugates for optical microscopy imaging under dark-field illumination has been confirmed with human HeLa cells and compared with a negative control using non-functionalized gold nanoparticles.

**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.