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nitbioconjugation

Datasheet

OligoLink Kit GOLD 30nm

Oligonucleotide functionalized gold nanoparticles are used in a wide range of applications, with the most common being as probes in DNA-based biosensing assays[1]. However, oligo-AuNPs could be also used for constructing nanoparticle superstructures based on DNA hybridization[2] or in therapy for nucleic acid delivery[3].

OligoLink Kit GOLD 30 nm is an easy-to-use gold nanoparticle conjugation kit for oligonucleotides. This kit guarantees the conjugation of your favorite oligonucleotide to high quality gold nanoparticles of 30 nm via a thiol group chemically introduced to either its 5´- or 3´-end. As thiols have very strong binding affinity for gold surfaces, a uniform and oriented coupling of the oligo onto the gold surface could be achieved by directly mixing the reduced thiol-modified oligonucleotide with the provided gold nanoparticles (**Figure 1**). The obtained compound results in a stable gold nanoparticle-oligonucleotide conjugate enable to bind complementary targets.

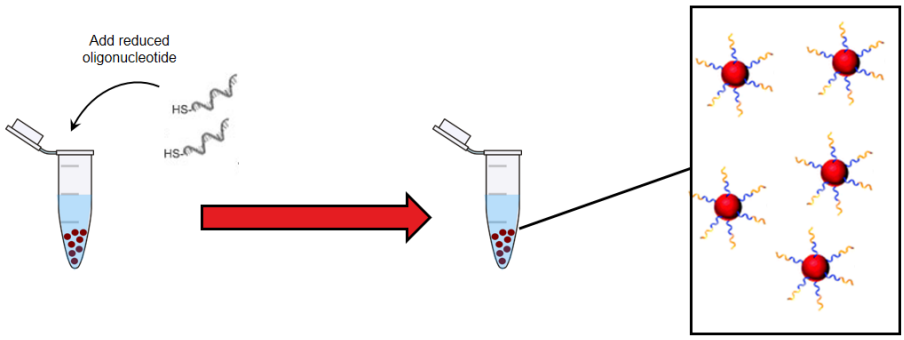


Figure 1. Scheme summarizing the overall conjugation process of the reduced thiolated-oligonucleotide of interest.

OligoLink Kit GOLD 30 nm has been designed in order to optimize the efficiency of the conjugation reaction in terms of ability to conjugate oligonucleotides of different lengths (between 10 and 140 mer) in the shortest time of reaction (1 hour and 10 minutes hands-on time). Besides, optimal oligonucleotide surface densities are suggested for oligonucleotide of different lengths (see available protocol).

1. Formats

Applicable to the following formats:

Ref No 03000418B – OligoLink Kit GOLD 30 nm – 1 mini reaction (50 µL, 20 OD)

Ref No 03000418C – OligoLink Kit GOLD 30 nm – 3 mini reactions (3 x 50 µL, 20 OD)

Ref No 03000418D – OligoLink Kit GOLD 30 nm – 1 midi reaction (500 µL, 20 OD)

2. Product Features

- **Gold nanoparticles with narrow size distribution:** A key feature for the use of oligo-AuNPs in Life Science or Materials Science is to work with stable AuNPs and with a narrow size distribution. As it could be observed in **Figure 2**, Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and UV-Vis spectroscopy analysis show the narrow size distribution of the AuNPs provided with the Kit.

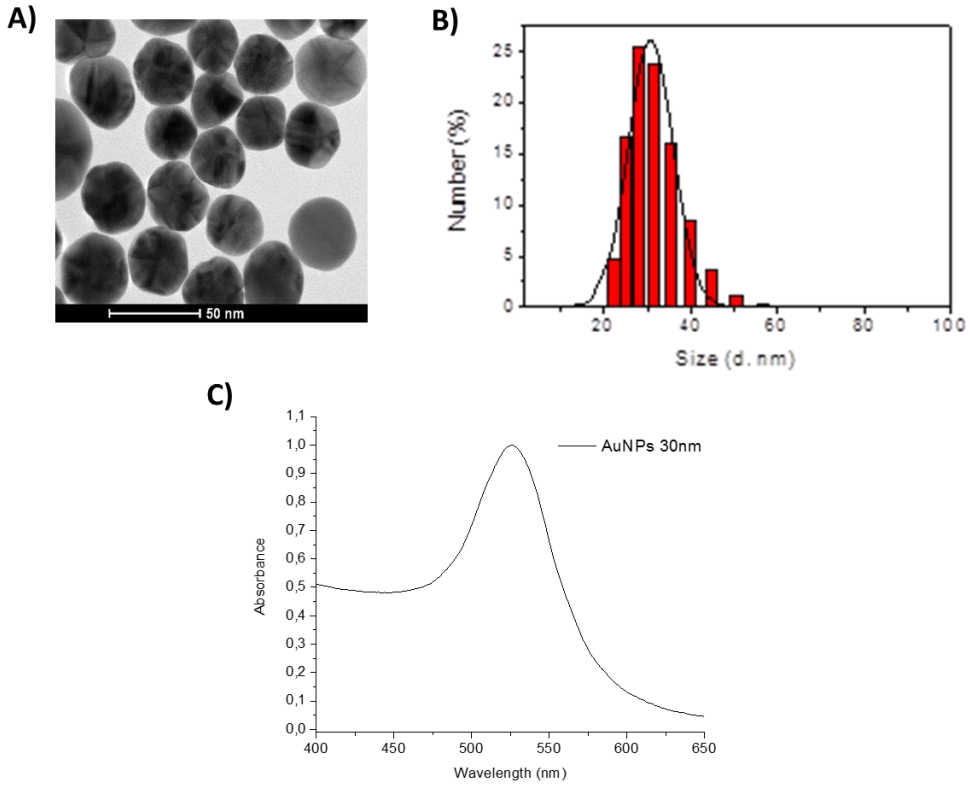
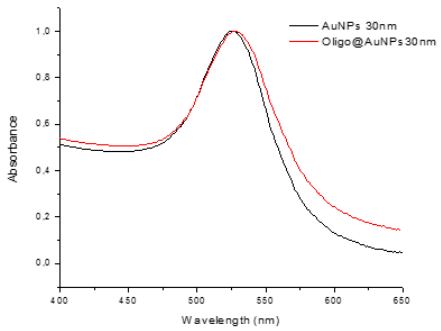


Figure 2. (A) TEM image, (B) size distribution expressed in number measured by DLS and (C) UV-Vis spectra of the AuNPs included.

- **Versatile and efficient oligonucleotide bioconjugation. OligoLink Kit GOLD 30 nm** allows the conjugation of oligonucleotides of different lengths within the range of 10 to 140 mer.

The binding of oligo with different lengths (40, 80 and 140 mer) shifted as expected the SPR band but do not significantly change the size distribution of the NPs (**Figure 3A**). Besides, the increase in net negative charge of all the obtained oligo@AuNPs conjugates, in comparison with the AuNPs provided with the kit, clearly indicates that oligo conjugation has took place (**Figure 3B**).

A)



B)

Sample	Z potential (mV)
AuNPs	-46
40 mer_oligo@AuNPs	-84
80 mer_oligo@AuNPs	-85
140 mer_oligo@AuNps	-85

Figure 3. **A)** UV-Vis spectra of AuNPs functionalized with a 140 mer oligo (red line) and AuNPs provided with the Kit (black line). Similar results were obtained for 40 and 80 pb oligo_@AuNPs; **B)** Z potential measurements at pH 7 of the AuNPs functionalized or not with the oligos of different lengths.

To confirm the binding of the selected oligos, the oligo@AuNPs conjugates were incubated in the presence of dithiotreitol (DTT). The supernatants obtained after centrifugation were analysed by agarose gel electrophoresis. As it can be observed in **Figure 4**, it was possible to release the bound oligos in all the cases which also confirms oligo conjugation.

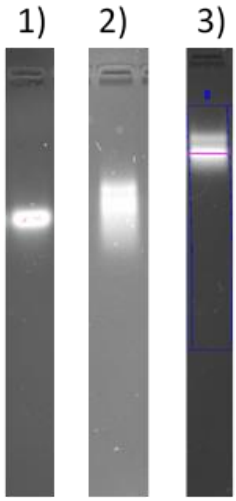


Figure 4: 2.5 % (w/v) agarose gel analysis of the supernatants obtained after treatment of oligo@AuNPs with DTT. Supernatant obtained from: 40 mer_oligo@AuNP (Lane 1); 80 mer_oligo@AuNP (Lane 2); and 140 mer_oligo@AuNP conjugates (Lane 3). The concentration of oligo added during conjugation was respectively of: 15 µM for 40 mer and 80 mer_oligo@AuNP and 30 µM for 140 mer_oligo@AuNP. Gel was run at 80 V constant in 0.5X TBE buffer for 30 minutes in the presence of GelRed™, and visualized under UV light.

Further, all oligo@AuNPs conjugates bind to a complementary oligo strand. This was analyzed by fluorescence spectroscopy using complementary oligonucleotides labelled with a NIR fluorescent dye. Each conjugate was incubated with different amounts of its complementary oligonucleotide. The fluorescent emission spectra of the nanoparticles was recorded after removal of the excess of complementary oligonucleotide that did not bind by several washing steps. As an example the fluorescent spectra obtained with 40 mer_oligo@AuNPs are showed in **Figure 5**. The spectra clearly indicate that the conjugate is able to bind increasing concentrations of the complementary fluorescent-labeled oligonucleotide.

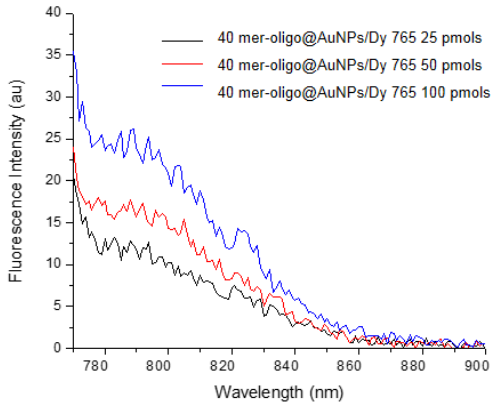


Figure 5: Fluorescent emission spectra after incubation of the 40 mer oligo@AuNP oligo-conjugates with different amounts of its corresponding fluorescent labelled complementary oligonucleotide.

The ability of each conjugate to bind a complementary oligo strand was also confirmed by dis-hybridization of the complementary strand and analysis by agarose gel electrophoresis. **Figure 6** shows the dis-hybridization achieved after boiling each hybridized conjugate in the presence of NaOH.

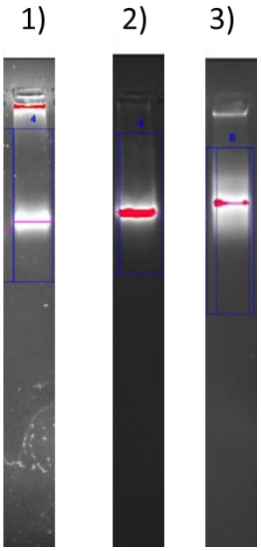


Figure 6: 2.5% (w/v) agarose gel analysis of the supernatants obtained after boiling the hybridized oligo@AuNPs in the presence of NaOH. Supernatant obtained from: 40 mer oligo@AuNP (Lane 1); 80 mer_oligo@AuNP (Lane 2); and 140 mer_oligo@AuNP conjugates (Lane 3). The concentration of complementary oligo added during hybridization was of 50 pmol for 40 mer and 80 mer_oligo@AuNP, and 100 pmol for 140 mer_oligo@AuNP. Gel was run at 80 V constant in 0.5X TBE buffer for 30 minutes minutes in the presence of GelRed™, and visualized under UV light.

3. How to use it?

A standard protocol for conjugation of thiol-modified oligonucleotides using our OligoLink Kit GOLD 30 nm is available.

4. Ordering information

Product Name	Size	Catalog No.
OligoLink Kit GOLD 30 nm_S	50 µl (20 OD)	03000418B
OligoLink Kit GOLD 30 nm_M	100 µl (20 OD)	03000418C
OligoLink Kit GOLD 30 nm_L	500 µl (20 OD)	03000418D

5. References

1. - "Plasmonic nanomaterials for biodiagnostics" Chem. Soc. Rev. (2014); 43(11), 3835-3853.

2. - "DNA assembly of nanoparticle superstructures for controlled biological delivery and elimination" Nature Nanotechnology (2014); 9, 148–155.

3. - "Gold Nanoparticles for Nucleic Acid Delivery" Molecular Therapy (2014); 22(6), 1075–1083.

6. Customer Service and Technical Support

For assistance or additional technical information, please contact us:

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Email: customer_support@nanoimmunotech.es

Product disclaimer

This nitbioconjugation product is to be used for research purposes only. Unless stated in the documentation of on an individual product label, catalog or other information provided to the buyer, IT IS FORBIDDEN TO USE IT for different purposes, including but not limited to them: in vitro diagnostic, use in food, pharmaceutical purposes, medical purposes, or use in cosmetic products, neither for use in humans nor animals, nor for any commercial purposes. Please refer to www.nitbioconjugation.com for the Material Safety Data Sheet of the product.