

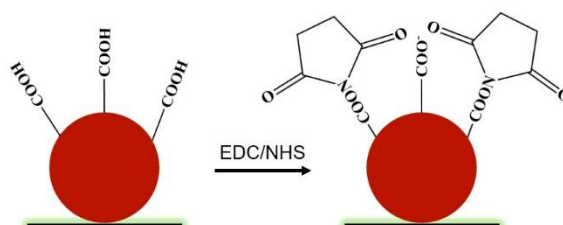
# Standard and Sensor Chip NanoAu-MM

## Customization Receptor Ligand Immobilization via Amine Coupling

Standard Chip NanoAu-MM and Sensor Chip NanoAu-MM offer great versatility for users to customize unique chips specific to their needs. The customization process involves functionalization of the carboxyl sensing surface with receptor ligands via amine coupling through a solution of EDC and NHS and includes three steps: surface activation, receptor ligand immobilization, and surface deactivation. The covalent bond between the sensing surface and receptor ligand ensures reproducible and uniform immobilization of the ligand.

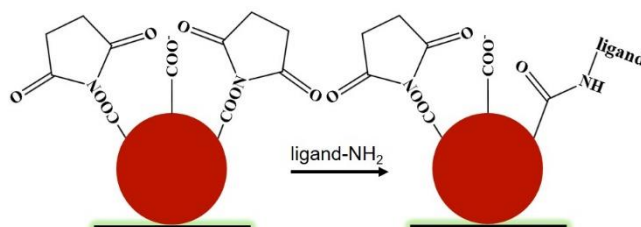
### Step 1 Surface activation

A solution of EDC and NHS activates the carboxyl group at the sensing surface through creating an intermediate NHS ester complex to react with the primary amine of receptor ligands.



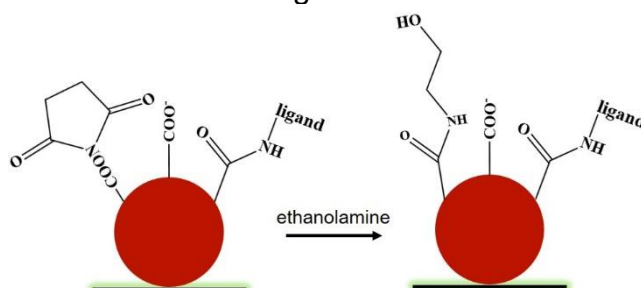
### Step 2 Receptor ligand immobilization

Receptor ligands, brought in proximity to the sensing surface, reacts with the NHS ester complex through the primary amine group. Receptor ligands with nucleophilic groups can generally be immobilized onto the carboxyl sensing surface.



### Step 3 Surface deactivation

A solution of ethanolamine converts the unreacted ester groups at the sensing surface to amide groups to deactivate the sensing surface.



**Note:** EDC stands for *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride while NHS stands for *N*-hydroxysuccinimide.

Table 1 displays the signal ratios of ten anti-TNF- $\alpha$  immobilizations with Sensor Chip NanoAu-MM. The mean of these ten immobilization runs was 0.08043 with a CV of 4.62%. This demonstrates the reproducible customization of sensor chips and is a prerequisite for reliable analyte detection.

**Table 1** Signal ratios, mean, and CV of ten anti-TNF- $\alpha$  immobilizations.

|              | 1       | 2       | 3       | 4       | 5       | 6       | 7       | 8       | 9       | 10      |
|--------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| signal ratio | 0.08363 | 0.08617 | 0.08211 | 0.08087 | 0.07987 | 0.07446 | 0.08112 | 0.08333 | 0.07661 | 0.07612 |
| mean         | 0.08043 |         |         |         |         |         |         |         |         |         |
| CV (%)       | 4.62    |         |         |         |         |         |         |         |         |         |

### Protocol

1. Surface wetting  
Deionized water, 80  $\mu$ L, 10 min
2. Surface activation  
Reagent 1, 80  $\mu$ L, 10 min, 2 times (total 20 min)  
**Note:** For preparation of Reagent 1, please refer to the INB Amine Coupling Reagent Preparation Protocol.  
  
Surface wash: deionized water, 80  $\mu$ L, 1 time  
Surface wash: buffer solution, 80  $\mu$ L, 1 time
3. Receptor ligand immobilization  
Receptor ligand solution, 80  $\mu$ L, 40 to 60 min  
  
Surface wash: buffer solution, 80  $\mu$ L, 2 times (solutions should be added consecutively)
4. Surface deactivation  
Reagent 2, 80  $\mu$ L, 7 min  
Note: Reagent 2 is provided in the amber tube found in the Standard Chip NanoAu-MM or Sensor Chip NanoAu-MM box.  
  
Surface wash: buffer solution, 80  $\mu$ L, 3 times (solutions should be added consecutively)
5. Surface equilibrium  
Buffer solution, 80  $\mu$ L, 10 min

### Considerations for Receptor Ligands

For successful receptor ligand immobilization and analyte detection, some general considerations regarding the receptor ligand are recommended:

1. Monoclonal
2. PBS-based, pH 7.0~7.4, carrier-free preferred (if available)
3. Previously used in ELISA
4. Conjugate-free (i.e. receptor ligand should not be labeled with dyes, beads, or other compounds)