πCode™ MicroDisc, Pre-activated User Manual

REF 80132 20,000 discs

RUO For Research Use Only

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IMPORTANT:
Read the instructions carefully prior to use

1. INTENDED USE

Pre-activated πCode MicroDisc is intended for the coupling of proteins, amine-modified nucleic acids, or any molecules containing primary amino group, onto the surface of the microdisc. It is ready to use and allows users to conveniently create their own assays using their own proteins, antibodies or nucleic acid probes. The product is for research use only, it is not intended for the diagnosis, prevention or treatment of human diseases.

2. INTRODUCTION

The PlexBio's carboxyl functionalized πCode MicroDiscs are designed for robust coupling of proteins, nucleic acids, and amine-containing molecules via an amide bond formation. Using a proprietary chemistry, we create a relatively stable intermediate acylating agent on the carboxyl functionality of microdisc as a "pre-activated" form that reacts readily with the amino groups of the ligands or probes to form a stable amide bond. The Pre-activated πCode MicroDisc is ready to use for the conjugation of proteins or nucleic acids of interest, circumventing the need to perform coupling reaction that is usually tedious and difficult to reproduce quantitatively.

In a typical assay using this system, the coupled πCode MicroDisc is used to capture the biotinylated target via the surface bound probe or ligand and stained with streptavidin-phycoerythrin (SA-PE) in the following incubation. This fluorescent complex can be analyzed on PlexBio™ 100 Fluorescent Analyzer. The intensity of the fluorescent signal is generally directly proportional to the amount of target on the surface of πCode MicroDisc.

The product of the πCode MicroDisc is provided at scales of $2x10^4$ discs per vial. The protein-/ nucleic acid-coupled πCode MicroDisc offers a wide variety of multiplexing bioassays that can be analyzed simultaneously by PlexBio's πCode technology.

3. WARNINGS AND PRECAUTIONS

- For research use only.
- This product should be used by qualified laboratory personnel only.
- Do not use the πCode MicroDisc past its expiration date.
- All chemicals, biological materials and human origin samples should be considered as potentially hazardous and/or infectious and should be treated accordingly.
- Store the πCode MicroDisc according to the product label and instructions.
- Do not mix πCode MicroDiscs from different lots.
- Dispose of unused reagents and waste according to applicable central/federal, state, and local regulations.
- General laboratory precautions should be taken:
  - Do not pipette by mouth.
  - Wear protective clothing (e.g., disposable powderless gloves and laboratory coats) and eye protection.
  - Do not eat, drink or smoke in the laboratory.
  - Wash hands thoroughly after handling samples and reagents.
- Material Safety Data Sheets (MSDS) are available upon request from PlexBio Customer Service.

4. STORAGE AND STABILITY

The shipping temperature for the πCode MicroDisc, Pre-activated is at 2-8°C, and the MicroDiscs should be frozen at -20°C upon arrival. The product is guaranteed up to the expiration date found on the label if handled properly.
Once πCode are coupled with proteins or nucleic acids, the stability is largely dependent on the characteristic of the coupled proteins or nucleic acids.

5. MATERIALS AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Micropipettes
- Filtered Pipette Tips
- Vortex mixer
- Micro-centrifuge (Max RCF: 2000g)
- Rotator (PlexBio Cat No. 80036; 42*12.5*16.8 cm)
- Magnetic Stand (for 0.5mL) (PlexBio Cat No. 80014)
- πCode Coupling Buffer Set (PlexBio Cat No. 80214)
- IntelliPlex™ 1000 πCode Processor (PlexBio Cat No. 80033)
- PlexBio 100 Fluorescent Analyzer (PlexBio Cat No. 80000)
- Lab oven (with adequate capacity to operate Rotator)
- 100% Methanol (for protein and small scale coupling)
- 0.5 mL SuperClear Screw Cap Microtubes (If needed; LABCON; Cat No.3640-870-000)

6. RECOMMENDED PROTOCOL

BUFFER PREAPRATION:

Note: All the buffers required with an exception of 100% Methanol, are provided in the πCode Coupling Buffer Set (PlexBio; Cat No. 80214).

If users choose to prepare their own buffers, please follow the instructions below to ensure the quality of coupling.

BUFFER FORMULATION-

Blocking Buffer:
- Tris buffered saline (TBS): 50mM Tris-HCl (PH 7.2), 150mM NaCl, 1% BSA, 0.1% NP40, 0.05% NaN_3

Rinse Buffer:
- 1% NP40 in H_2O

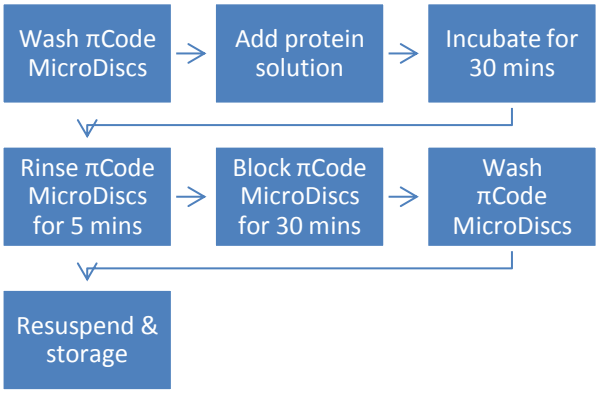
Wash Buffer:
- PBST: PBS containing 0.1% Tween 20, 0.01% NaN_3

Storage Buffer:
- PBS containing 0.1% BSA, 1mM EDTA, 50% Glycerol, 0.05% NaN_3

PROTEIN COUPLING NOTES

- This protocol is optimized for coupling of proteins.
- **Protein Solution** :
  i. Suggested protein concentration for coupling is approximately **40 µg/mL**. However, because of the large diversity of protein compositions, the optimal protein concentrations should be determined individually for each protein.
  ii. Do not dilute the protein in **Tris buffer** or **any amine-containing buffer**.
  iii. The protein solution should be free of sodium azide, glycerol, or any other amine-containing additives.
- The stability of the protein-coupled πCode MicroDiscs is largely dependent on the intrinsic characteristics of the protein.
- This protocol is recommended for coupling 20,000 πCode MicroDisc in a vial.

PROTEIN COUPLING WORKFLOW



PROTEIN COUPLING PROCEDURE

1. Add 200 µL **ice-cold 100% Methanol** to reconstitute the pre-activated πCode MicroDisc.
2. Vortex the tube for 30 seconds.
3. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
4. Place the tube into the magnetic stand (for 0.5mL; Cat No. 80014) by the position of inner tube to be visible. Invert the stand back and forth to let the activated πCode MicroDisc coagulate at the tube sidewall evenly.
5. Under the magnetic separation, remove the supernatant without disrupting the activated πCode MicroDiscs.

6. Repeat step 1-5.
7. Open the tube cap and heat the πCode MicroDiscs at 37 °C for 3 minutes to evaporate the remaining Methanol.
8. Add 200 µL **Protein Solution** to the πCode MicroDisc, and vortex the tube for 30 seconds.
9. Mix on a rotator (PlexBio Cat No. 80036) by setting a program at "MODE- U2 and RPM-99" for 30 minutes at room temperature.
10. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
11. Add 20 µL **Rinse Buffer** to the protein coupled πCode mixture, and mix on a rotator using program at "MODE- U2 and RPM-99" for 5 minutes at room temperature.
12. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
13. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the bubbles first (if needed) and following by removing the supernatant.
14. Wash the coupled πCode MicroDisc with 200 µL **Blocking Buffer**.
15. Vortex for 30 seconds.
16. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
17. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
18. Add 200 µL **Blocking Buffer** and mix on a rotator using program at "MODE- U2 and RPM-99" for 30 minutes at room temperature.
19. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
20. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
21. Wash the coupled πCode MicroDiscs with 200 µL **Wash Buffer**.
22. Vortex for 30 seconds.
23. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
24. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
25. Repeat step 21-24.
26. Add 200 µL **Storage Buffer** to make the coupled πCode MicroDiscs at 100 MD/µL.
27. Store the coupled πCode MicroDiscs at 2-8°C.

SMALL SCALE PROTEIN COUPLING PROCEDURE (OPTIONAL)

1. Add 200 µL **ice-cold 100% Methanol** to reconstitute the pre-activated πCode MicroDisc at 100 MD/µL.
2. Vortex for 30 seconds.
3. Dispense 10 to 20 µL to a new 0.5mL screw cap microtube (1,000-2,000 MD/tube).

NOTE-
The unused reconstituted πCode MicroDisc solution can be stored in 100% Methanol at -20°C for up to one week.

4. Add 100 µL **Methanol (100%)** to the tube.
5. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
6. Place the tube into the magnetic stand (for 0.5mL; Cat No. 80014) by the position of inner tube to be visible. Invert the stand back and forth to let the activated πCode MicroDisc coagulate at the tube sidewall evenly.
7. Under the magnetic separation, remove the supernatant without disrupting the activated πCode MicroDiscs.
8. Repeat step 4-7.
9. Open the tube cap and heat the πCode MicroDiscs at 37 °C for 3 minutes to evaporate the remaining Methanol.
10. Add 100 µL **Protein Solution** to the πCode MicroDisc, and vortex the tube for 30 seconds.
11. Mix on a rotator (PlexBio Cat No. 80036) by setting a program at "MODE- U2 and RPM-99" for 30 minutes at room temperature.
12. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
13. Add 10 µL **Rinse buffer** to the protein coupled πCode mixture, and mix on a rotator using program at "MODE- U2 and RPM-99" for 5 minutes at room temperature.
14. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
15. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the bubbles first (if needed) and following by removing the supernatant.

16. Wash the coupled πCode MicroDisc with 100 µL **Blocking Buffer**.
17. Vortex for 30 seconds.
18. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
19. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
20. Add 100 µL **Blocking Buffer** and mix on a rotator using program at "MODE- U2 and RPM-99" for 30 mins at room temperature.
21. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
22. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
23. Wash the coupled πCode MicroDiscs with 100 µL **Wash Buffer**.
24. Vortex for 30 seconds.
25. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
26. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
27. Repeat step 23-26.
28. Add 10-20 µL **Storage Buffer** to make the πCode MicroDiscs at 100 MD /µL.
29. Store the πCode MicroDiscs at 2-8°C.

PROTEIN COUPLING CONFIRMATION PROTOCOL

The targets need to be biotin-labeled prior to assay. Alternatively, biotinylated antibodies can be used to specifically label the target in the subsequent incubation.

The conjugation relies on a covalent bond formed between carboxyl groups on the πCode MicroDisc and amino groups on the surface of the protein. The reaction may alter the activities or the structure of the coupled protein.

The performance of each protein probe used must be validated individually before proceeding to multiplex assay. It is also recommended to assess the coupling efficiency after the coupling process.

1. Prepare a working πCode MicroDisc solution by diluting the coupled πCode MicroDisc stocks to a final concentration of 5 Discs/µL in **Storage Buffer**.

   NOTE: *At least 20 µL of the working πCode MicroDisc solution is required for each reaction.*

2. Prepare a solution of biotinylated target protein or suitable antibody (depending on coupled protein) in PBS-1% BSA.

   NOTE: *The appropriate concentrations of the biotinylated target protein/antibody for the test depends on the affinities between the coupled protein and its target. For many immunoassays, target concentration of 0.1-5 µg/mL is usually a good starting point.*

3. Aliquot 20 µL of the πCode MicroDisc solution prepared in Step 1 into wells of the plate, respectively.
4. Add 50 µL of PBS-1% BSA, into a tested well as a blank sample.
5. Add 50 µL of the biotinylated target protein or suitable antibody solution prepared in Step 2 into another tested well.
6. Place the plate into IntelliPlex 1000 πCode Processor and build a customized program for following hybridization process. The program should include:
   - Suitable incubation condition for target protein.
   - Wash cycles.
   - SA-PE reaction.

   NOTE: *Please refer to the user manual of IntelliPlex 1000 πCode Processor for all necessary information. Contact Plexbio Customer Service if needed.*

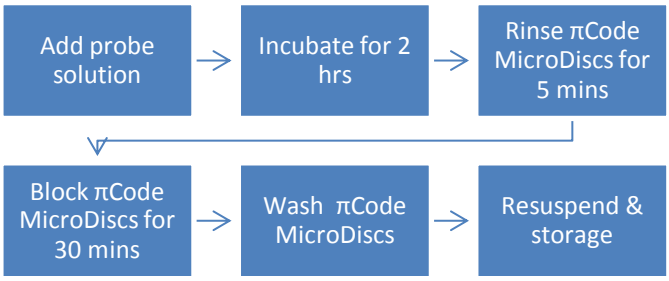
7. Place the plate into PlexBio 100 Fluorescent Analyzer for image recognition and fluorescent signal acquisition.

   NOTE: *Please refer to the user manual of PlexBio 100 Fluorescent Analyzer for all necessary information. Contact Plexbio Customer Service if needed.*

NUCLEIC ACID COUPLING NOTES

- Please make sure that the oligonucleotide (probe) to be coupled is **amine modified**.
- **Probe Solution:**
  i. Suggested DNA probe concentration for coupling is **3 µM in ddH_2O**. The optimal DNA probe concentrations should be determined individually for each DNA probe.
  ii. The probe solution should be free of sodium azide, glycerol, or any other amine-containing additives.
- The DNA -coupled πCode MicroDiscs could be stable up to 12 months in general.
- This protocol is recommended for coupling 20,000 πCode MicroDisc per vial.

NUCLEIC ACID COUPLING WORKFLOW



NUCLEIC ACID COUPLING PROCEDURE

1. Add 200 µL **Probe Solution** into πCode MicroDisc, Pre-activated, and vortex the tube for 30 seconds.
2. Mix on a rotator (PlexBio Cat No. 80036) using program at "MODE- U2 and RPM-99" for 2 hours at 37°C (in a lab oven).
3. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
4. Add 20 µL **Rinse Buffer** to the probe coupled πCode mixture, and mix on a rotator using program at "MODE- U2 and RPM-99" for 5 minutes at 37°C (in a lab oven).
5. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
6. Place the tube into the magnetic stand (for 0.5mL; Cat No. 80014) by the position of inner tube to be visible. Invert the stand back and forth to let the activated πCode MicroDisc coagulate at the tube sidewall evenly.
7. Under the magnetic separation, remove bubbles first (if needed) and continue to remove supernatant with a transfer pipette without disrupting the activated πCode MicroDiscs.
8. Wash the coupled πCode MicroDisc with 200 µL **Blocking Buffer**.
9. Vortex for 30 seconds.
10. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
11. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
12. Add 200 µL **Blocking Buffer** and mix on a rotator using program at "MODE- U2 and RPM-99" for 30 minutes at 37°C (in a lab oven).
13. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
14. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
15. Wash the πCode MicroDiscs with 200 µL **Wash Buffer**.
16. Vortex for 30 seconds.
17. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
18. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
19. Repeat step 15-18.
20. Add 200 µL **Storage Buffer** to make the πCode MicroDiscs at 100 MD/µL.
21. Store the πCode MicroDiscs at 2-8°C.

SMALL SCALE NUCLEIC ACID COUPLING PROCEDURE (OPTIONAL)

1. Add 200 µL **ice-cold 100% Methanol** to reconstitute Pre-activated πCode MicroDisc.
2. Vortex the πCode MicroDisc for 30 seconds.
3. Dispense 10 to 20 µL to a new 0.5mL screw cap microtube (1,000-2,000 MD/tube).

NOTE-
The unused reconstituted πCode MicroDisc solution can be stored in 100% Methanol at -20 ˚C for up to one week.

4. Add 100 µL **Probe Solution** into the tube, and vortex the tube for 30 seconds.
5. Mix on a rotator using program at "MODE- U2 and RPM-99" for 2 hours at 37°C (in a lab oven).
6. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
7. Add 10 µL **Rinse Buffer** to the probe coupled πCode mixture, and mix on a rotator using program at "MODE- U2 and RPM-99" for 5 minutes at 37°C (in a lab oven).
8. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
9. Place the tube into the magnetic stand (for 0.5mL; Cat No. 80014) by the position of inner tube to be visible. Invert the stand back and forth to let the activated πCode MicroDisc coagulate at the tube sidewall evenly.
10. Under the magnetic separation, remove bubbles first (if needed) and continue to remove supernatant with a transfer pipette without disrupting the activated πCode MicroDiscs.
11. Wash the coupled πCode MicroDisc with 100 µL **Blocking Buffer**.
12. Vortex for 30 seconds.
13. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
14. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
15. Add 100 µL **Blocking Buffer** and mix on a rotator using program at "MODE- U2 and RPM-99" for 30 minutes at 37°C (in a lab oven).
16. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
17. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
18. Wash the πCode MicroDiscs with 100 µL **Wash Buffer**.
19. Vortex for 30 seconds.
20. Briefly centrifuge for 10 seconds to collect all πCode MicroDiscs at the bottom.
21. Place the tube into the magnetic stand to coagulate πCode MicroDiscs to sidewall and remove the supernatant.
22. Repeat step 18-21.
23. Add 10-20 µL **Storage Buffer** to make the πCode MicroDiscs with 100 MD/µL.
24. Store the πCode MicroDiscs at 2-8°C.

NUCLEIC ACID COUPLING CONFIRMATION PROTOCOL

The target need to be biotin-labeled to yield fluorescent signal after SA-PE labeling. For PCR-based assay, using biotinylated primers of target sequences allows the amplicons to be biotin-labeled. For other approaches, please consult Plexbio Customer Service.

The performance of each DNA probe used must be validated individually before proceeding to multiplex assay. It is also recommended to assess the coupling efficiency after the coupling process.

1. Prepare a working πCode MicroDisc solution by diluting the coupled πCode MicroDisc stocks to a final concentration of 5 Discs/µL in **Storage Buffer**.

   NOTE: *At least 20 µL of the working πCode MicroDisc solution is required for each reaction.*

2. Prepare a solution of biotinylated oligonucleotide which is complementary to coupled DNA probe.

   NOTE: *Concentration may vary depending on the sequence or length. 10nM of biotinylated oligonucleotide in ddH_2O or TE buffer is usually feasible.*

3. Aliquot 20 µL of the πCode MicroDisc solution prepared in Step 1 into wells of the plate.
4. Add 100 µL of 5xSSPE or SSC into all tested wells.
5. Add 10 µL of ddH_2O into a tested well as a blank sample.

6. Add 10 µL of biotinylated oligonucleotide solutions prepared in Step 2 into another tested well.
7. Place the plate into IntelliPlex 1000 πCode Processor and build a customized program for following hybridization process. The program should include:
   - Suitable incubation condition for target and probe.
   - Wash cycles.
   - SA-PE reaction.

   NOTE: *Please refer to the user manual of IntelliPlex 1000 πCode Processor for all necessary information. Contact Plexbio Customer Service if needed.*
8. Place the plate into PlexBio 100 Fluorescent Analyzer for image recognition and fluorescent signal acquisition.

   NOTE: *Please refer to the user manual of PlexBio 100 Fluorescent Analyzer for all necessary information. Contact Plexbio Customer Service if needed.*

7. TROUBLESHOOTING GUIDE

The troubleshooting guide listed below addresses possible problem causes and solutions provided during coupling reaction and coupling confirmation.

Problem	Possible Cause	Recommendation
Low Disc Count	πCode MicroDisc were lost during coupling.	Make sure to use the recommended magnetic stand (please see chapter 5.) to collect πCode MicroDisc for all washes.
Low or no MFI signal	The detection target is not biotin-labeled.	Ensure the target is biotin-labeled during reaction. Increase the concentration of biotinylated ingredient. Verify the affinity between probe and biotinylated target.
	Interference in protein/probe solution.	Protein/probe solution should be free of sodium azide, glycerol, or any other amine-containing additives. Dialyze the stock solution if needed.
	Low concentration of protein/ probe solution.	Increase the concentration of protein/probe solution to optimize the coupling condition.
	Incorrect temperature of incubation during coupling.	Make sure the incubation is at room temperature for protein and 37°C for nucleic acid during coupling.
High MFI background	Unspecific binding during hybridization	Make sure the materials are not contaminated. Verify the protein/ probe design to avoid unspecific binding. Optimize the incubation condition of hybridization.
	Residual of target sample or SA-PE	Perform additional wash cycles. Make sure IntelliPlex 1000 πCode Processor is properly operated and maintained.

8. SYMBOLS

Symbol	Explanation	Symbol	Explanation
RUO	Research Use Only	REF	Catalog number
LOT	Batch number		Consult instructions for use
	Manufacturer		Use by Date
	Temperature limitation	Σ	Contains sufficient for <n> tests

Notice to User

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