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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Activated DNA (5x)

Catalog # 80605

Lot #: 150707 Conc.: 5x

DESCRIPTION: Activated DNA for PARP assay

APPLICATIONS: Study enzyme kinetics, and screen small molecular inhibitors of PARP for drug discovery and HTS applications

SUPPLIED AS: Aqueous buffer solution

QUANTITY: 500 μ l

STORAGE: -20°C or -80°C

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Assay Protocol:

Materials: Blocking buffer; Streptavidin-HRP, HRP substrate(s); Histone mixture; Activated DNA (BPS catalog number 80605); Assay buffer (BPS catalog number 80600); PARP Substrate (BPS catalog number 80601).

Step 1: Coat 50 μ l of histone solution to a 96 well plate (VWR catalog no. 62409-300)

1. Dilute 5x histone mixture 1:5 with PBS.
2. Add 50 μ l of histone solution to each well and incubate at 4°C overnight.
3. Wash the plate three times with PBST buffer (1x PBS containing 0.05% Tween 20).
4. Block the wells by adding 150 μ l of Blocking buffer to every well. Incubate at room temperature for 30 min.
5. Wash plate three times with PBST buffer as above.

Step 2: Ribosylation reaction

1. Run the reaction in a total volume of 50 μ l containing 100-300 ng of PARP, 2.5 μ l of 10x assay mixture, 2.5 μ l of 10x PARP buffer, and 5 μ l of activated DNA at room temperature for 1 hour.
2. Wash plate three times with PBST buffer as above.

Step 3: Detection

1. Dilute Streptavidin-HRP 1:500 in Blocking buffer.
2. Add 50 μ l of diluted Streptavidin-HRP to each well, incubate for 30 min. at room temperature.
3. Wash three times with PBST buffer as above.
4. Just before use, mix on ice 50 μ l HRP chemiluminescent substrate A and 50 μ l HRP chemiluminescent substrate B and add 100 μ l per well.

Step 4: Immediately read sample in a microtiter-plate reading chemiluminescence.

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