Nucleosome Remodeling Assay by EpiDyne-FRET

EpiDyne-FRET: a functionalized recombinant nucleosome-based assay platform for chromatin remodeling studies

Chromatin remodeling, or the repositioning of nucleosomes, regulates DNA access and thus gene expression and genome repair. Many ATP-dependent remodeling enzyme complexes are associated with human disease but are challenging study targets due to the requirement for nucleosome-based substrates. EpiCypher has addressed this need by developing the EpiDyne platform of fully recombinant remodeling substrates to monitor nucleosome repositioning along DNA using Fluorescence Resonance Energy Transfer (FRET) readout (Figure 1).

**FIGURE 1**

EpiDyne-FRET Nucleosome Remodeling Substrates consist of a Cy5-labeled human histone octamer (H2A T120C-Cy5; shown as red section of octamer) wrapped by 5' Cy3-labeled DNA (217bp; green ball) comprising a terminally nucleosome positioning sequence (147bp Widom 601 adjacent to a TG-GA-repeat region refractory to nucleosome assembly. In its assembled starting state, Cy3-Cy5 FRET is at a maximum. The activity of an ATP-dependent remodeler (e.g. RSC or another SWI/SNF ATPase) is detected by a reduction in FRET signal as the Cy3-labeled DNA 5' end is moved away from the Cy5-labeled octamer. EpiDyne-FRET is a one-step no-wash method immediately compatible with HTS applications.

**Useful for**

- Inhibitor screening and development
- Structure-Activity Relationship assays
- Biochemical profiling of ATPase family proteins
Aberrant nucleosome organization can severely disrupt gene expression, DNA repair and cellular differentiation, and it also plays a major role in human disorders, including cancer, inflammation, autoimmunity, schizophrenia, cardiovascular disease, and intellectual disability. Remarkably, nearly 20% of all cancers contain mutations in subunits from the SWI/SNF family of ATP-dependent chromatin remodeling complexes. These enzyme complexes regulate local genome access by ‘pumping’ the DNA around histone octamers, thus ‘sliding’ nucleosomes.

Recurrent somatic mutations in SWI/SNF subunits are observed in multiple cancers, supporting a driver role in tumorigenesis. The mutated remodeling proteins are attractive therapeutic targets, since further compromising their ATPase activity promotes cancer cell death but spares normal cells.

<table>
<thead>
<tr>
<th>SUBUNIT</th>
<th>CANCER</th>
</tr>
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<tbody>
<tr>
<td>ARID1A</td>
<td>Ovarian, Hepatocellular, Bladder, Gastric, Endometrioid, Pancreatic, Colon, Lung, Neuroblastoma, Burkitt Lymphoma</td>
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<tr>
<td>ARID1B</td>
<td>Melanoma, Neuroblastoma, Hepatocellular, Pancreatic, Liver</td>
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<tr>
<td>PBRM1</td>
<td>Renal cell carcinoma, Breast, Gastric, Pancreatic</td>
</tr>
<tr>
<td>ARID2</td>
<td>Melanoma, Hepatocellular, Pancreatic</td>
</tr>
<tr>
<td>SMARCA2</td>
<td>Lung, Colon, Breast</td>
</tr>
<tr>
<td>SMARCA4</td>
<td>Lung, Medulloblastoma, Burkitt Lymphoma, SCCOHT</td>
</tr>
<tr>
<td>SMARCB1</td>
<td>Rhaboid tumor, Familial Schwannomatosis</td>
</tr>
<tr>
<td>SMARCE1</td>
<td>Spinal meningitis</td>
</tr>
<tr>
<td>BRD7</td>
<td>Breast</td>
</tr>
</tbody>
</table>

TABLE 1

List of cancers associated with various SWI/SNF subunit mutations. Asterisks in Figure 2 indicate associated cancers in Table 1.
EpiDyne-FRET allows unprecedented access to disease-relevant ATP-dependent chromatin remodeling complexes

**FIGURE 3**

EpiDyne-FRET nucleosomes (20 nM) were incubated with RSC (10 nM) in the presence of fixed 2 mM ATP with increasing amounts of ATPγS. Upon ATP addition, reactions were immediately read in an Envision Multi-label plate reader. Data is presented as the mean of the Cy3-Cy5 ratio (N=2).

**ATPγS titration with fixed ATP**

**IC₅₀ calculation for ATPγS**

**FIGURE 4**

Data from Figure 4 (20-minute time point) were analyzed to determine the IC₅₀ value for ATPγS (2.29 mM). Data is presented as the mean of the Cy3-Cy5 ratio vs. the ATPγS concentration.

![Graphs showing ATPγS titration and IC₅₀ calculation](image)

Multiple HTS-compatible EpiDyne assays formats available (see Related Products below). Inquire at info@epicypher.com

**ORDERING INFO**

**EpiDyne-FRET Nucleosome**
Cat. No. 16-4201
Price: $549 / 50 µg

**RELATED PRODUCTS**

**EpiDyne Nucleosome / Chromatin Remodeling Assay Substrate ST601-GATC1**
Cat. No. 16-4101
Price: $499 / 50 µg

**EpiDyne Nucleosome / Chromatin Remodeling Assay Substrate ST601-GATC1, Biotinylated**
Cat. No.: 16-4111
Price: $499 / 50 µg

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