**BRIC Kit**

**5-Bromouridine Immunoprecipitation Chase**

- The optimum kit for analyzing stability/half-life of mRNA and non-coding RNA (ncRNA).
- Useful tools to identify novel ncRNA and transcripts based on their half-lives.

- For characterization of mRNA and ncRNA
- For searching new ncRNA biomarkers and drug target
- For discovering new RNA degradation control pathways

Transcriptional inhibitors such as actinomycin D have been used in the analysis of stability and half-life of RNA; however, the use of transcriptional inhibitors alters the stability and localization of RNA, and it has been shown to interfere with the results of analysis.

**BRIC Kit** enables determination of RNA stability by chasing chronological decreases of BrU-labeled RNA under physiologically undisturbed conditions. In the BRIC protocol, cells are pulse-labeled with BrU for constant period and washed with PBS to remove the BrU-containing medium, and then cells are chronologically harvested, followed by preparation of total RNA including newly BrU-labeled RNA. The BrU-labeled RNA can be specifically immunoprecipitated with Anti-BrdU mAb provided by MBL, followed by isolation of BrU-labeled RNA from immunocomplex on carrier material, such as protein G magnetic beads. The isolated BrU-labeled RNA can be analyzed to determine its own stability and half-life by various methods in molecular biology – RT-qPCR, deep sequencing or microarray.

*Reference*

**Analytical methods that can be applied following BRIC**

- Deep sequencing
- RT-qPCR
- Microarray

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HeLa, K562, 293T, Jurkat, HEK293 and T-47D cells were pulse-labeled with 150 μM BrU for 24 hours. Then, the cells were washed and harvested at chase time 0 and 24 hours. After RNA extraction, BrU-labeled RNA was isolated by BRIC Kit. The isolated BrU-labeled RNAs were analyzed by RT-qPCR. The RNAs derived from housekeeping genes such as 18S rRNA and ACTB were stable in most of cell lines, while HIF-1α and ADM were unstable. The labeling efficiency was low in Jurkat, HEK293 and T-47D cells, which indicates labeling efficiency varies depending on the cell lines.

HeLa cells were pulse-labeled with 150 μM BrU for 24 hours. Then, cells were washed and harvested at chase time 0, 4, 8, 12 and 24 hours. After RNA extraction, BrU-labeled RNA was isolated by BRIC Kit. Isolated BrU-labeled RNA was analyzed by RT-qPCR. As expected, the transcripts derived from housekeeping genes, such as 18S rRNA and ACTB, showed relatively long half-lives, while HIF-1α and ADM* showed much shorter half-lives.

*ADM gene encodes a potent hypotensive peptide which plays important roles in both normal and disease conditions.

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<th>Size</th>
<th>Storage temp.</th>
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<td>BRIC Kit</td>
<td>20 assays</td>
<td>-20°C</td>
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<tr>
<td>RN1008*</td>
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*RN1007 and RN1008 are sold as a set. RN1007 and RN1008 should be stored at different temperature.

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**Kit components:**

**Code RN1008 (Storage temp. 2-8°C):**

1. Wash buffer: 41 mL × 3 bottles
2. mi-Solution I: 240 μL × 1 vial: enzyme solution
3. mi-Solution II: 5.6 mL × 1 vial: diluent for Solution I
4. mi-Solution III: 3.6 mL × 1 vial: protein dissolvent
5. mi-Solution IV: 90 μL × 1 vial: co-precipitator Solution IV can increase RNA precipitation efficiently.
6. Protein G-Magnetic beads: 1.5 mL × 4 vials 1% beads slurry (mouse IgG binding capacity: 7 μg/mg beads)

**Code RN1007 (Storage temp. -20°C):**

8. BrU solution (100 mM): 1.1 mL × 2 vials
9. Anti-BrdU mAb: 450 μL × 1 vial
10. Spike-in control: 80 μL × 1 vial

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