

Kinase Assay Using Radioisotope (Autoradiography)

Preparation of assay conditions, a 20 μ L reaction containing the following:

- ✓ 20 mM HEPES KOH (pH7.5)
- ✓ 10 mM MgCl₂
- ✓ 1 mM DTT
- ✓ 100 μ M [gamma ³²P] ATP (0.5 μ Ci)
- ✓ 4 μ L of Abnova's enzyme and substrate cocktail (2 μ g each of histone H1, histone H3, MBP and beta casein)

Step-by-step procedure:

1. Start the reaction by adding 4 μ L of [gamma ³²P] ATP (0.5 μ Ci)
2. Incubate for 60 minutes at 30°C.
3. Terminate the reaction by adding 7 μ L of 4X sample buffer.
4. Subsequently boil the sample.
5. Analyze the 15 μ L of sample on SDS-PAGE gel.
6. Stain, destain, and then dry the gel.
7. Take an autoradiograph by exposing the gel for 24 hours.

Kinase substrates:

- ✓ H3 histone, family 3A - **calf thymus**
Molecular weight: approx. 16.5-17 kDa on SDS-PAGE
- ✓ Myelin basic protein - **bovine brain**
Molecular weight: approx. 21-22 kDa on SDS-PAGE
- ✓ Histone H1.0 - **calf thymus**
Molecular weight: approx. 32-33 kDa on SDS-PAGE
- ✓ Beta-casein protein- **bovine milk**
Molecular weight: approx. 33-34 kDa on SDS-PAGE



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- ✓ 20 mM HEPES KOH (pH7.5)
- ✓ 10 mM MgCl₂
- ✓ 1 mM DTT
- ✓ 0.1 mM EGTA
- ✓ 2.5 mM CaCl₂
- ✓ 250 ng/ml calmodulin
- ✓ 100 μ M [γ -³²P] ATP (0.5 μ Ci)
- ✓ 4 μ L of Abnova's enzyme and substrate cocktail (2 μ g each of histone H1, histone H3, MBP and beta casein)

Step-by-step procedure:

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- ✓ Histone H1.0 - **calf thymus**
Molecular weight: approx. 32-33 kDa on SDS-PAGE
- ✓ H3 histone, family 3A - **calf thymus**
Molecular weight: approx. 16.5-17 kDa on SDS-PAGE



Phosphatase Assay Using Fluorescent Substrates

Preparation of assay conditions, a 50 μ L reaction containing the following:

- ✓ 100 mM Tris HCl (pH 7.5)*
- ✓ 40 mM NaCl
- ✓ 1 mM DTT
- ✓ 0.1 mM OMFP (3-o-methylfluorescein phosphate)
- ✓ 5 μ L of Abnova's enzyme in each well of a 96-well microplate

Step-by-step procedure:

1. Measure fluorescence intensity of sample for 60 minutes at 5-minute intervals using a microtiter plate fluorometer with excitation at 485 nm and emission at 535 nm at 30°C.
2. Measure and calculate the rate of reaction while the velocity of the reaction remains constant.

*50 mM Mops KOH pH 6.0 for acid phosphatase.

Equipment(s):

- ✓ Microtiter plate fluorometer (Arvo HTS; Perkin Elmer)