Kinase Assay Using Radioisotope (Autoradiography)

Preparation of assay conditions, a 20µL reaction containing the following:
20 mM Hepes KOH (pH7.5)
10 mM MgCl2
1 mM DTT
100 µM [gamma 32P] ATP (0.5µCi)
4 µL of Abnova's enzyme and substrate cocktail (2 µg each of histone H1, histone H3, MBP and beta casein)

Step-by-step procedure:
1. Start the reaction by adding 4 µL of [gamma 32P] ATP (0.5µCi)
2. Incubate for 60 minutes at 30°C.
3. Terminate the reaction by adding 7 µL of 4X sample buffer.
4. Subsequently boil the sample.
5. Analyze the 15 µL 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

Kinase Assay Using Radioisotope (Autoradiography)
Preparation of assay conditions, a 20µL reaction containing the following:
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 µM [gamma 32P] ATP (0.5µCi)
4 µL of Abnova's enzyme and substrate cocktail (2 µg each of histone H1, histone H3, MBP and beta casein)

Step-by-step procedure:
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2. Incubate for 60 minutes at 30°C.

Abnova Products are distributed in the UK and Ireland by Caltag Medsystems Ltd, Botolph Claydon, Buckingham, MK18 2LR, UK.

Tel: Freephone (UK) 0800 279 9113, or +44 (0) 1296 715459

Email: office@caltagmedsystems.co.uk Web: www.caltagmedsystems.co.uk

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Kinase substrates:
Beta-casein protein- **bovine milk**
Molecular weight: approx. 33-34 kDa on SDS-PAGE
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Molecular weight: approx. 21-22 kDa on SDS-PAGE
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 µL reaction containing the following:
100 mM Tris HCl (pH 7.5)*
40 mM NaCl
1 mM DTT
0.1 mM OMFP (3-o-methlfluorescein phosphate)
5 µL 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-minutes 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)