# Azoxymethane

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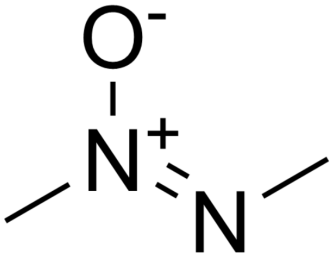
| **Cat. No.:** | HY-111375 |
| **CAS No.:** | 25843-45-2 |
| **Molecular Formula:** | $C_2H_6N_2O$ |
| **Molecular Weight:** | 74 |
| **Target:** | DNA Alkylator/Crosslinker |
| **Pathway:** | Cell Cycle/DNA Damage |
| **Storage:** | Solution, -20°C, 2 years |



## BIOLOGICAL ACTIVITY

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| **Description** | Azoxymethane is a colon carcinogen which leads to the formation of DNA adducts. |
| **In Vitro** | Azoxymethane is a colon carcinogen which leads to the formation of DNA adducts. On an equal protein basis, hepatic microsomes are much more active than SI and colon microsomes in NADPH-dependent Azoxymethane bioactivation and $N^7$-mG adduct formation. Hepatic microsomes show the highest activity in the hydroxylation of Azoxymethane, followed by SI and colon microsomes[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **In Vivo** | Azoxymethane can be used in animal modeling to construct tumor models.<br><br>Regardless of the strain, the amounts of $O^6$-mG and $N^7$-mG produced by Azoxymethane are highest in the liver, followed by proximal and distal colons, which have similar levels, and then by duodenum, jejunum and ileum. Results indicate that the Azoxymethane-induced DNA adduct formation in the SI and colon does not depend on bioactivation by hepatic P450 enzymes. Irrespective of the mouse strain, no aberrant crypt foci (ACF) is detected in the colons of saline-treated mice; in contrast, colonic ACF is detected in all three strains of Azoxymethane-treated mice[1]. The Azoxymethane-treated athymic mice have approximately an 11-fold lower tumor incidence than similarly treated WT animals[2].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |

## PROTOCOL

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| **Kinase Assay** [1] | The assay for Azoxymethane-induced in vitro DNA adduct formation is performed. Briefly, microsomes (0.5 to 2.0 mg/mL) are incubated with calf thymus DNA (1 mg/mL) and Azoxymethane (200 μM) in a total volume of 1.0 mL. The assay buffer consists of 0.1 M Tris-HCl (pH 7.4), 1 mM EDTA, 20 mM $MgCl_2$, 0.3 M KCl, and 1.5 mM NADPH. Incubations are carried out at 37°C for 60 min in a shaking water bath. An additional 30 nM of NADPH is added after the first 30 min. The reaction is stopped by the addition of 0.5 mL of ice-cold 7.5 M ammonium acetate. DNA is then extracted for tissue homogenates. Control incubations are performed without NADPH[1].<br>MCE has not independently confirmed the accuracy of these methods. They are for reference only. |
| **Animal Administration** [1] | Male, 8 to 10 week old, WT-A/J, IECN-A/J, and LCN-A/J mice (8 per group) are treated with either saline or Azoxymethane (7.5 mg/kg BW, s.c.), once weekly for 3 weeks. Mice are sacrificed 6 weeks post-treatment for aberrant crypt foci (ACF) detection. The entire colon is excised. A longitudinal incision is made along the entire length of the colon, which is further cut into two |

equal-length segments, representing proximal and distal portions of the colon. The segments are dipped in PBS to remove fecal pellets and then kept flat between filter papers in 10% buffered formalin for at least 24 h. Subsequently, the colons are immersed in freshly prepared 0.1% methylene blue for 10 min and rinsed briefly in deionized $H_2O$ to remove excess dye. The colon is mounted carefully on a microscope slide with the mucosal surface side up and viewed under a light microscope. The ACF in the entire mucosal surface of the colon are counted blindly and independently by two investigators and recorded [1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## CUSTOMER VALIDATION

- EBioMedicine. 2020 Nov;61:103068.
- Clin Transl Med. 2024 Jan;14(1):e1535.
- Phytomedicine. 2023 Sep 20, 155095.
- Free Radic Biol Med. 2021 Jun 15;S0891-5849(21)00377-4.
- Int Immunopharmacol. 2023 Nov 22:126:111188.

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## REFERENCES

[1]. Megaraj V, et al. Role of hepatic and intestinal p450 enzymes in the metabolic activation of the colon carcinogen azoxymethane in mice. Chem Res Toxicol. 2014 Apr 21;27(4):656-62.

[2]. Whetstone RD, et al. Colon carcinogenesis in wild type and immune compromised mice after treatment with azoxymethane, and azoxymethane with dextran sodium sulfate. Mol Carcinog. 2016 Jul;55(7):1187-95.



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