COSMO BIO CO., LTD.
Inspiration for Life Science

TransGenic Inc.

Code No.KAL-KH002

For research use only

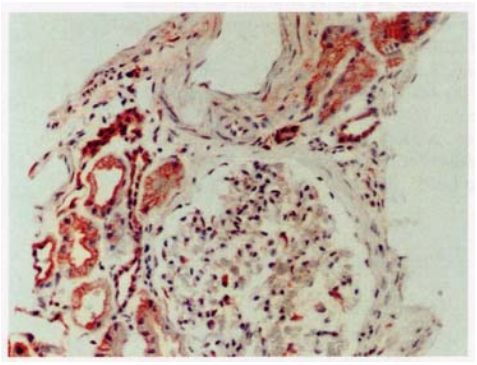
Advanced Glycation End Products （AGEs）

Anti AGEs Monoclonal Antibody（Clone No. 6D12） Fab', Peroxidase conjugated

Reaction of protein amino groups with glucose leads, through the early products such as a Schiff base and Amadori rearrangement products, to the formation of advanced glycation end products (AGEs). Recent immunological studies using anti-AGEs antibody (6D12) demonstrated the presence of AGEs-modified proteins in several human tissues: (ⅰ) human lens (nondiabetic and noncataractous), (ⅱ) renal proximal tubules in patients with diabetic nephropathy and chronic renal failure, (ⅲ) diabetic retina, (ⅳ) peripheral nerves of diabetic neuropathy, (ⅴ) atherosclerotic lesions of arterial walls, (ⅵ) β 2-microglobulin forming amyloid fibrils in patients with hemodialysis-related amyloidosis, (ⅶ) senile plaques of patients with Alzheimer's disease, (ⅷ) the peritoneum of CAPD patients, (ⅸ) skin elastin in actinic elastosis, and (ⅹ) ceriod/lipofuscin deposits. These results suggest a potential role of AGEs-modification in normal aging as well as age-enhanced disease processes. This antibody named as 6D12 has been used to demonstrate AGEs-modified proteins in these human tissues, indicating potential usefulness of this antibody for histochemical identification and biochemical quantification of AGEs-modified proteins.

Package Size	20µg (200µL/vial)
Format	Mouse monoclonal antibody, Peroxidase conjugated 0.1 mg/mL
Buffer	Block Ace as a stabilizer, containing 0.1%Proclin as bacteriostat
Storage	Store below –20℃ Once thawed, store at 4℃. Repeated freeze-thaw cycles should be avoided.
Clone No.	6D12
Subclass	IgG1
Purification Method	The splenic lymphocytes from BALB/c mouse, immunized with AGEs-BSA were fused to myeloma P3U1 cells. The hybrid cells were screened, and the cell line (6D12) with positive reaction to AGEs-human serum albumin but negative to BSA was selected through successive subclonings and grown in ascitic fluid of BALB/c mouse, from which the anti-AGEs antibody was purified by Protein G affinity chromatography (Reference No.1) and conjugated.

Working dilution for immunohistochemistry: 2µg /mL; for ELISA: 0.1-0.5µg /mL



Immunohistochemical staining of renal proximal tubules and glomeruli in patients with diabetic nephropathy, using anti-AGEs antibody 6D12

Yamada, K. et al,.

Clinical nephrology, Vol.42, 354-361, 1994

Immunohistochemical staining of th eary stage of human athrosclerotic lesions of the aorta with anti-AGEs antibody 6D12.

Kume, S. et al,

American Journal of Pathology, Vol.147, 654-667, 1995

COSMO BIO CO., LTD.
Inspiration for Life Science

TransGenic Inc.

Code No.KAL-KH002

Advanced Glycation End Products （AGEs）

Anti AGEs Monoclonal Antibody（Clone No. 6D12）Fab', Peroxidase conjugated

【Specificity】

The initial study (Ref. 1) revealed that 6D12 does not recognize early products (Schiff base and Amadori products), but shows a positive reaction to AGEs-samples obtained either from proteins, lysine derivatives or monoamino-carboxylic acids, indicating the immunospecificity to a common structure among AGEs-structures. The subsequent study (Ref. 10) revealed of 6D12 is an N^{ε} - carboxymethyllysine(CML)-protein adduct.

【Reference】

1. Horiuchi, S.et al.: Immunochemical approach to characterize advanced glycation end products of the Maillard reaction; Evidence for the presence of a common structure. J. Biol. Chem. 266: 7329, 1991. **2**. Araki, N. et al.: Immunochemical evidence for the presence of advanced glycation end products in human lens proteins and its positive correlation with aging. J. Biol. Chem. 267: 10211, 1992. **3**. Miyata, T. et al.: β_2-Microglobulin modified with advanced glycation end products is a major component of hemodialysis-associated amyloidosis. J. Clin. Invest. *92*: 1243, 1993. **4**. Yamada, K et al.: Immunohistochemical study of human advanced glycosylation end-products (AGE) in chronic renal failure. Clin. Nephrol. *42*: 354, 1994. **5**. Kume, S. et al.: Immunohistochemical and ultrasturactural detection of advanced glycation end products in atherosclerotic lesions of human aorta using a novel specific monoclonal antibody. Am. J. Pathol. 147 : 654, 1995. **6**. Makino, H. et al.: Ultrastructure of nonenzymatically grycated mesangial matrix in diabetic nephropathy. Kidney International 48: 517, 1995. **7**. Mori, T. et al.: Localization of advanced grycation end products of Maillard reaction in bovine tissues and their endocytosis by macrophage scavenger receptors. Exp. Molec. Pathol. 63:135, 1995 **8**. Miyata, T. et al.: Identification of pentosidine as a native structure for advanced glycation end products in β_2-Microglobulin forming amyloid fibrils in patients with dialysis-related amyloidsis. Proc. Natl. Acad. Sci. USA. 93: 2353, 1996 **9**. Kimura, T. et al.: Accumulation of advanced glycation end products of the Maillard reaction with age in human hippocampal neurons. Neurosci. Lett. 208: 53,1996. **10**. Ikeda, K. et al.: N^{ε}-(carboxymethyl) lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. Biochemistry 35: 8075,1996. **11**. Horiuchi, S. et al.: AGE modified proteins and their potential relevance to atherosclerosis. Trends Cardiovasc. Med. 6: 163, 1996. **12**. Hammes, H-P et al.: Modification of vitronectin by advanced glycation alters functional properties in vitro and in the diabetic retina. Lab. Invest. 75: 325, 1996. **13**. Kimura, T. et al.: Identification of advanced grycation end products of the Maillard reaction in Pick's disease. Neurosci. Lett. 219: 95, 1996. **14**. Nakayama, M. et al.: immunohistochemical detection of advanced grycosylation end-products in the peritoneum and its possible pathophysiological role in CAPD. Kidney Intentional 51: 182, 1997. **15**. Mizutani, K. et al.: Photo-enhanced modification of human skin elastin in actinic elastosis by N^{ε}-(carboxymethyl)lysine, one of the glycoxidation products of the Maillard reaction. J. Invest. Dermatol. 108: 797, 1997. **16**. Murata, T. et al.: The relationship between expression of advanced glycation end products and vascular endothelial growth factor in human diabetic retinas. Diabetologia 40: 764, 1997. **17**. Sugimoto, K. et al.: Localization in human diabetic peripheral nerve of N^{ε}-carboxymethyllysine-protein adducts, one of advanced glycation endproducts. Diabetologia 40: 1380, 1997. **18**. Shimokawa, I. Et al.: Advanced glycosylation end-products in adrenal lipofuscin. J. Gerontol. 51A: B49, 1998. **19**. Yoshida, S. et al.: Immunohistochemical study of human advanced glycation end-products and growth factors in cardiac tissues of patients on maintenance dialysis and with kidney transplantation. Clin. Nephrol.49: 273, 1998. **20**. Matsuse, S. et al.: immunohistochemical localisation of advanced glycation end products in pulmonary fibrosis. J. Clin. Pathol, 51:515,1998

Distributor

COSMO BIO CO., LTD.
Inspiration for Life Science

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618

人と科学のステキな未来へ
コスモ・バイオ株式会社

Trans Genic Inc.

Code No.KH002

研究用試薬

Advanced Glycation End Products （AGEs）

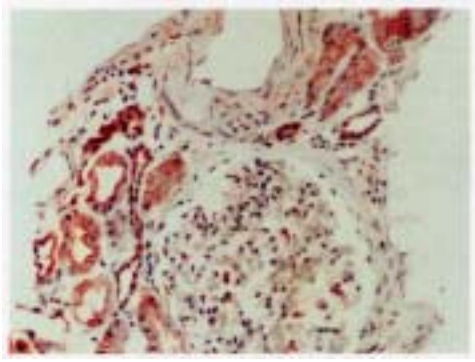
抗 AGEs モノクローナル抗体（Clone No. 6D12）
Fab’ , peroxidase conjugated

AGEs(Advanced Glycation End Products)は、タンパク質の非酵素的糖付加反応（メイラード反応）により、シッフ塩基、アマドリ転移生成物（前期生成物）を経由し、脱水、酸化、縮合などの複雑な反応を受けて形成される最終生成物です。AGEs は、蛍光・褐色・分子架橋形成などの特徴の他、AGEs 受容体により認識されるという生化学的特性を有しています。

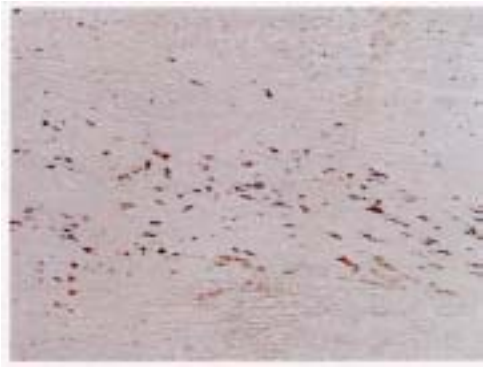
近年の抗 AGEs 抗体による解析の結果、(1)ヒト水晶体（加齢に伴う増加）、(2)糖尿病性腎症や慢性腎不全患者の腎近位尿細管、(3)糖尿病患者の網膜、(4)糖尿病性神経障害患者の末梢神経、(5)粥状動脈硬化病変部、(6)透析性アミロイドーシスのβ2-マイクログロブリン、(7)アルツハイマー病患者の老人斑、(8)CAPD 患者の腹膜、(9)弾力線維症の皮膚のエラスチン、(10)セロイド／リポフスチン沈着部位などに AGEs が蓄積することが分かってきました。これらの知見は、老化自体や老化に伴う慢性疾患に AGEs が深く関与していることを示唆しています。

本抗体(6D12)は、加齢に伴う慢性疾患の研究に非常に有用であると思われます。

容量	20µg（200µL/vial）
形状	マウスモノクローナル抗体 Peroxidase 標識 0.1mg/mL、凍結品
バッファー	PBS [2%ブロックエース（安定化蛋白）、0.1% proclin 含有]
保管方法	－20℃以下 抗体を低濃度で冷蔵保管されますと、失活する恐れがあります。 融解後は 4℃で保存し、お早めにご使用下さい。 凍結融解を繰り返すことは避けてください。
クローン番号	6D12
サブクラス	IgG1
製造方法	AGEs-BSA で免疫した BALB/c マウスの脾臓細胞とマウスミエローマ P3U1 を融合して得たハイブリドーマを BALB/c マウス腹腔内で増殖させ、腹水を採取。 採取した腹水より Protein G アフィニティーカラムにて精製後、標識。
使用濃度	組織染色：2µg/mL ELISA：0.1～0.5µg/mL



糖尿病性腎症患者の腎近位尿細管および糸球体
Yamada, K. et al,.
Clinical nephrology, Vol.42, 354-361, 1994



粥状動脈硬化　初期病変
Kume, S. et al,
American Journal of Pathology, Vol.147, 654-667, 1995

Trans Genic Inc.

Code No.KH002

Advanced Glycation End Products （AGEs）

抗 AGEs モノクローナル抗体（Clone No. 6D12）Fab’, peroxidase conjugated

【特異性】

6D12 は反応前期生成物（シッフ塩基やアマドリ転移生成物）には反応しませんが、タンパク質やリジン誘導体またはモノカルボン酸から生じた AGEs には反応性を示すことが確認されています（参考文献 **1**）。

6D12 のエピトープはタンパク質中のリジンが修飾されて生じる N-カルボキシメチルリジン（CML）であることが示されました（参考文献 **10**）。

【参考文献】

1. Horiuchi, S.et al.: Immunochemical approach to characterize advanced glycation end products of the Maillard reaction; Evidence for the presence of a common structure. J. Biol. Chem. 266: 7329, 1991. **2**. Araki, N. et al.: Immunochemical evidence for the presence of advanced glycation end products in human lens proteins and its positive correlation with aging. J. Biol. Chem. 267: 10211, 1992. **3**. Miyata, T. et al.: β_2-Microglobulin modified with advanced glycation end products is a major component of hemodialysis-associated amyloidosis. J. Clin. Invest. *92*: 1243, 1993. **4**. Yamada, K et al.: Immunohistochemical study of human advanced glycosylation end-products (AGE) in chronic renal failure. Clin. Nephrol. *42*: 354, 1994. **5**. Kume, S. et al.: Immunohistochemical and ultrasturactural detection of advanced glycation end products in atherosclerotic lesions of human aorta using a novel specific monoclonal antibody. Am. J. Pathol. 147 : 654, 1995. **6**. Makino, H. et al.: Ultrastructure of nonenzymatically grycated mesangial matrix in diabetic nephropathy. Kidney International 48: 517, 1995. **7**. Mori, T. et al.: Localization of advanced grycation end products of Maillard reaction in bovine tissues and their endocytosis by macrophage scavenger receptors. Exp. Molec. Pathol. 63:135, 1995 **8**. Miyata, T. et al.: Identification of pentosidine as a native structure for advanced glycation end products in β_2-Microglobulin forming amyloid fibrils in patients with dialysis-related amyloidsis. Proc. Natl. Acad. Sci. USA. 93: 2353, 1996 **9**. Kimura, T. et al.: Accumulation of advanced glycation end products of the Maillard reaction with age in human hippocampal neurons. Neurosci. Lett. 208: 53,1996. **10**. Ikeda, K. et al.: N^{ε}-(carboxymethyl) lysine protein adduct is a major immunological epitope in proteins modified with advanced glycation end products of the Maillard reaction. Biochemistry 35: 8075,1996. **11**. Horiuchi, S. et al.: AGE modified proteins and their potential relevance to atherosclerosis. Trends Cardiovasc. Med. 6: 163, 1996. **12**. Hammes, H-P et al.: Modification of vitronectin by advanced glycation alters functional properties in vitro and in the diabetic retina. Lab. Invest. 75: 325, 1996. **13**. Kimura, T. et al.: Identification of advanced grycation end products of the Maillard reaction in Pick’s disease. Neurosci. Lett. 219: 95, 1996. **14**. Nakayama, M. et al.: immunohistochemical detection of advanced grycosylation end-products in the peritoneum and its possible pathophysiological role in CAPD. Kidney Intentional 51: 182, 1997. **15**. Mizutani, K. et al.: Photo-enhanced modification of human skin elastin in actinic elastosis by N^{ε}- (carboxymethyl)lysine, one of the glycoxidation products of the Maillard reaction. J. Invest. Dermatol. 108: 797, 1997. **16**. Murata, T. et al.: The relationship between expression of advanced glycation end products and vascular endothelial growth factor in human diabetic retinas. Diabetologia 40: 764, 1997. **17**. Sugimoto, K. et al.: Localization in human diabetic peripheral nerve of N^{ε}-carboxymethyllysine-protein adducts, one of advanced glycation endproducts. Diabetologia 40: 1380, 1997. **18**. Shimokawa, I. Et al.: Advanced glycosylation end-products in adrenal lipofuscin. J. Gerontol. 51A: B49, 1998. **19**. Yoshida, S. et al.: Immunohistochemical study of human advanced glycation end-products and growth factors in cardiac tissues of patients on maintenance dialysis and with kidney transplantation. Clin. Nephrol.49: 273, 1998. **20**. Matsuse, S. et al.: immunohistochemical localisation of advanced glycation end products in pulmonary fibrosis. J. Clin. Pathol, 51:515,1998

人と科学のステキな未来へ
コスモ・バイオ株式会社

〒135-0016　東京都江東区東陽 2-2-20 東陽駅前ビル
URL：http://www.cosmobio.co.jp/
● 営業部（お問い合わせ）
TEL：（03）5632-9610　FAX：（03）5632-9619
TEL：（03）5632-9620