●TOYOBO ANTIBODIES●

(Diagnostic Reagent Grade)

ANTI BIOTIN MONOCLONAL ANTIBODY

from Mouse hybridoma

PREPARATION and SPECIFICATION

Appearance	: Solution with PBS buffer containing 0.05% NaN_3
Grade	: Grade Ⅱ
Immunogen	: Biotin-KLH
Purity	: ≥90%(GPC)

PROPERTIES

Stability	: Stable at 4℃ and −20℃ for at least 12 months	(Fig.1)
Isotype	: IgG_1	

APPLICATIONS

This monoclonal antibody is useful as a trap reagent for biotin-labeled material such as antibodies, DNA probes and allergens. (Fig.2, 3, 4)

Examples

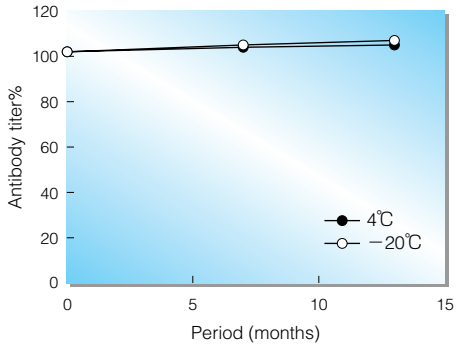

Fig.1. Stability (Liquid form)

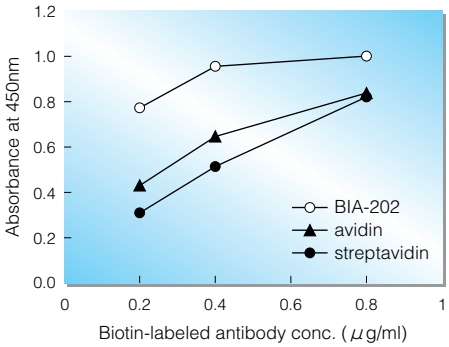

Fig.2. BIA-202-Avidin: Comparison of trapping performance on CEA two step sandwich EIA using biotin-labeled antibody

BIA-202, avidin, or streptavidn coated 96-well plates was prepared as biotin-trap 96-well plates, respectively. 30ng/ml CEA solution was assayed by two step sandwich EIA using biotin-trap 96-well plates, biotin-labeled anti CEA antibody and HRP-labeled anti CEA antibody. The higher absorbance was observed in use of the BIA-202 coated plate than in use of avidin coated plate.

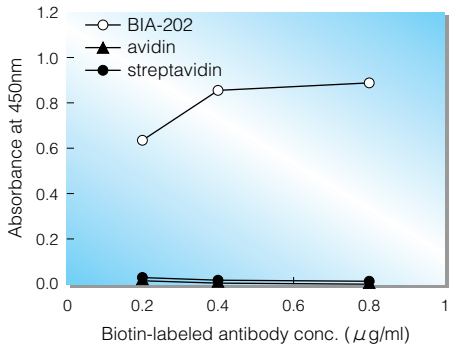

Fig.3. BIA-202-Avidin: Comparison of trapping performance on CEA two step sandwich EIA using biotin-labeled antibody in the presence of D-biotin

30ng/ml CEA solution containing 2mg/L D-biotin was assayed by the method mentioned in Fig.2. The concentration-dependent response curve of biotin-labeled antibody was observed in use of the BIA-202 coated plate.

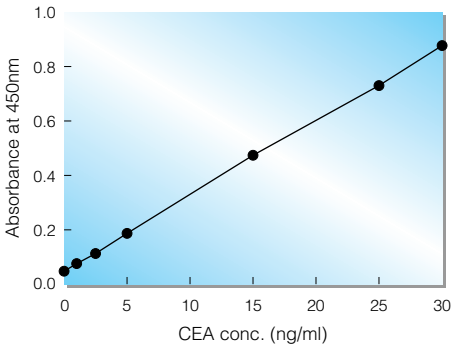

Fig.4. CEA one step sandwich EIA with BIA-202 coated 96-well polystyrene plate

CEA solution was assayed by one step sandwich EIA using BIA-202 coated 96-well plates, biotin-labeled anti CEA antibody and HRP-labeled anti CEA antibody. Liner CEA-dose response curve was observed.

Examples in detail

Fig.2. BIA-202-Avidin: Comparison of trapping performance on CEA two step sandwich EIA using biotin-labeled antibody

Biotin-trap 96-well plates was prepared as shown below. 50uL of 0.01mg/ml BIA-202, avidin, or streptavidn in 0.1M phosphate buffer pH7.0 were added to 96-well polystyrene plate and incubated at 25℃ for 2 hr, respectively. After discarding these solution, 300uL of blocking buffer was added to the plates and incubated at 25℃ for 1 hr.

After washing PBS-T, 25ul of 30ng/ml CEA solution and 25ul of 0.2, 0.4, 0.8ug/ml biotin-labeled anti-CEA monoclonal antibody were added and incubated at 37℃ for 1hr, respectively. After washing PBS-T, 50ul of HRP-labeled anti-CEA monoclonal antibody were added to the plate and incubated at 37℃ for 1 hr. After washing PBS-T, 50ul of TMB solution was added to the plate and incubated at room temperature for 10 min. After 50ul of 1N sulfuric acid solution was added to the plate, absorbance of each well was measured at 450nm.

The higher absorbance was observed in use of the BIA-202 coated plate than in use of avidin coated plate.

Fig.3. BIA-202-Avidin: Comparison of trapping performance on CEA two step sandwich EIA using biotin-labeled antibody in the presence of D-biotin

30ng/ml CEA solution containing 2mg/L D-biotin was assayed by the method mentioned in Fig.2.

The concentration-dependent response curve of biotin-labeled antibody was observed in use of the BIA-202 coated plate.

Fig.4. One step sandwich EIA with BIA-202 coated 96-well polystyrene plate

Biotin-trap 96-well plates were prepared as shown Fig.2. After washing PBS-T, 25ul of the standard solution (hCEA) and 25ul of pre-mixed conjugate solution (containing 0.4ug/ml biotin-labeled anti-CEA monoclonal antibody and HRP-labeled anti-CEA monoclonal antibody) were added to the plate and incubated at 37℃ for 1 hr. After washing PBS-T, the enzyme reaction and the measurement were done as shown Fig.2.

Liner CEA-dose response curve was observed.

実施例説明（Japanese）

Fig.2. BIA-202-Avidinにおける捕捉特性の比較: ビオチン標識抗体を使ったCEA 2ステップ サンドイッチ EIA

ビオチン捕捉 96穴プレートを以下のように調製した。：0.1Mリン酸バッファーpH7.0で0.01mg/mℓになるよう希釈したBIA-202、アビジン、ストレプトアビジンをそれぞれ50 μL 96穴ポリスチレンプレートに添加し25℃で2Hr放置した。液を廃棄した後、ブロッキングバッファー300 μL添加し25℃で1Hr放置した。

PBS-Tで洗浄後、30ng/mℓのCEA溶液25 μlと0.2、0.4、0.8 μg/mℓ各濃度のビオチン標識抗CEAモノクローナル抗体25 μlを添加し、37℃で1時間放置した。PBS-Tで洗浄後、HRP標識抗CEAモノクローナル抗体50 μlを添加し37℃1時間放置した。PBS-Tで洗浄した後、TMB溶液50 μlを添加し10分室温で反応、その後1N硫酸で反応を止め、450nmの吸光度を測定した。

BIA-202を使ったプレートではアビジン同等以上の高い吸光度が得られた。

Fig.3. BIA-202-Avidinにおける捕捉特性の比較: Dービオチン共存下でのビオチン標識抗体を使ったCEA 2ステップ サンドイッチ EIA

2mg/L D-ビオチンを含んだ30ng/mℓ CEA溶液を使い、Fig.2.記載の方法に従って測定を行なった。

BIA-202を使ったプレートで、ビオチン標識抗体濃度依存的な応答曲線が得られた。

Fig.4. BIA-202を固相化した96穴ポリスチレンプレートを使った1ステップ EIA

ビオチン捕捉96穴プレートをFig.2.記載の方法により調製した。PBS-Tで洗浄後、CEA標準液25 μlと0.4 μg/mℓビオチン標識抗CEA抗体とHRP標識抗CEAモノクローナル抗体の混合液25 μlを添加し、37℃にて1時間放置した。PBS-Tで洗浄後、Fig.2.記載の酵素反応と測定を実施した。

直線的なCEAドーズ応答カーブが得られた。