

CODE No. 5333

For Research Use Only, Not for use in diagnostic procedures.

Ab-Match Assembly Mouse Neuropilin-1 (CD304) kit

For technical material or related information, please refer to <https://ruo.mbl.co.jp/product/cancer/abmatch.html>.

■ Advantage

By using Ab-Match Assembly Mouse Neuropilin-1 (CD304) kit in combination with the Ab-Match Universal kit (MBL; code no. 5310), mouse neuropilin-1 can be measured quantitatively and specifically. The two kits are sufficient to produce one 96-well microplate. This product is intended for research use only.

■ Materials provided 1 × 96 wells

| Name | Materials | Quantity | Dilution ratio |
|---|--|-----------------|----------------|
| Mouse Neuropilin-1 standard | Recombinant mouse Neuropilin-1* (lyophilized powder) | 1 vial | 1:10 – 1:640 |
| Anti-mouse Neuropilin-1 capture antibody | Rat anti-mouse Neuropilin-1 monoclonal antibody (50% Glycerol/PBS) | 60 µL × 1 vial | 1:200 |
| Anti-mouse Neuropilin-1 detection antibody | Biotin conjugated rat anti-mouse Neuropilin-1 monoclonal antibody | 110 µL × 1 vial | 1:101 |
| Streptavidin conj. peroxidase | Streptavidin conjugated peroxidase (SA-HRP) | 110 µL × 1 vial | 1:101 |

* Reconstitute “Standard” by dissolving lyophilized Mouse Neuropilin-1 with distilled water. The volume of distilled water is specific for each lot, and it is indicated in the label on the tube of “Mouse Neuropilin-1 standard.”

To avoid repeated freezing and thawing, prepare aliquots.

Note: Kit components must be brought to room temperature (20-30°C) prior to use.

■ Protocol

| Coating step | Blocking step | Sample incubation step | Detection antibody incubation step | SA-HRP step | Substrate incubation step | Reading |
|---|--|--|--|--|---|--|
| Add 100 µL diluted capture antibody to each well ↓ Incubate over night at 2-8°C | Wash 2 times ↓ Add 200 µL of Blocking Agent to each well ↓ Incubate 1 hour at room temperature | Dump out the contents of the wells ↓ Add 100 µL of samples or standards to each well ↓ Incubate 1 hour at room temperature | Wash 4 times ↓ Add 100 µL of diluted detection antibody to each well ↓ Incubate 1 hour at room temperature | Wash 4 times ↓ Add 100 µL of diluted SA-HRP to each well ↓ Incubate 30 minutes at room temperature | Wash 4 times ↓ Dump out the contents of the wells ↓ Add 100 µL of Substrate Solution to each well ↓ Incubate 30 minutes at room temperature | Add 100 µL of Stop Solution to each well ↓ Read absorbance at 450 nm |

■ Storage and Stability

All kit components must be stored at -20 ~ -30°C. All reagents are stable for 12 months after shipment.

■ Related product

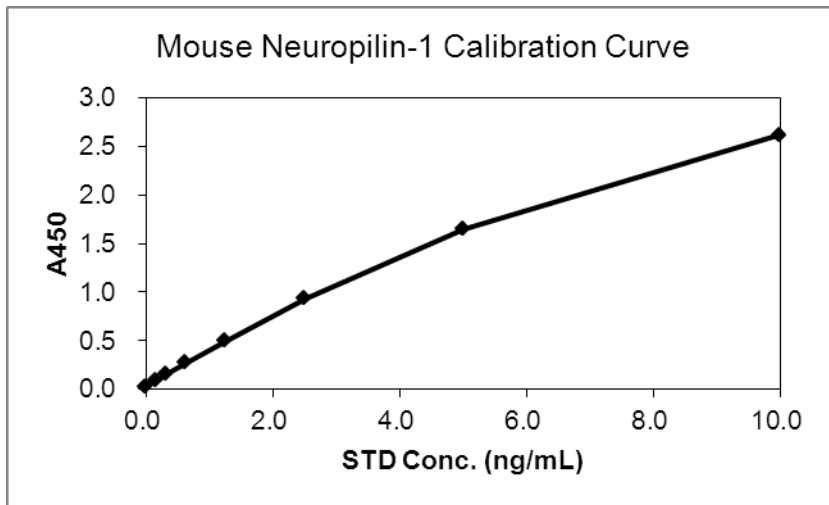
5310 Ab-Match Universal kit

■ References

- 1) Beck, B., *et al.*, *Nature* **478**, 399-403 (2011)
- 2) Bruder, D., *et al.*, *Eur. J. Immunol.* **34**, 623-630 (2004)
- 3) He, Z. and Tessier-Lavigne, M., *Cell* **90**, 739-751 (1997)
- 4) Kawakami, A., *et al.*, *J. Neurobiol.* **29**, 1-17 (1996)

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■ **Calibration curve**



■ **References data**

1. Intra-assay

| ng/mL | CV % |
|-------|------|
| 0.94 | 3.2 |
| 2.96 | 1.4 |
| 3.85 | 2.4 |
| 5.84 | 3.1 |

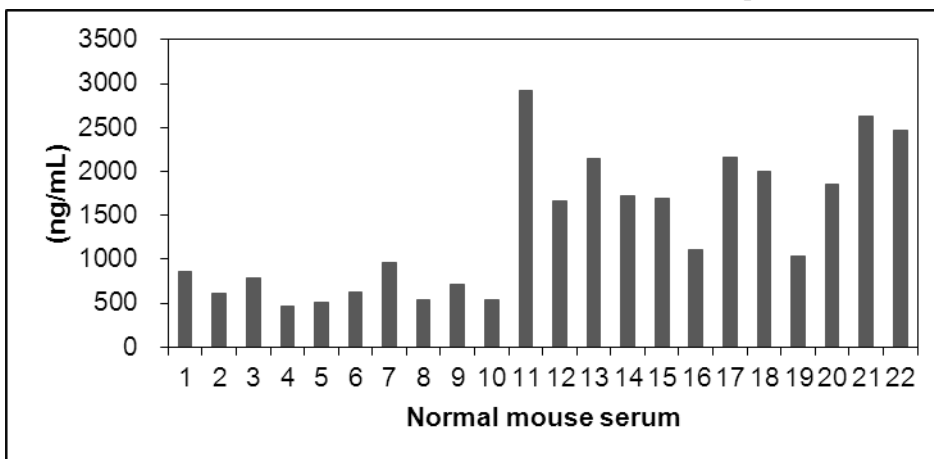
2. Detection limit

| | |
|--------------------------|-------------|
| LOB (Limit of Blank) | 0.051 ng/mL |
| LOD (Limit of Detection) | 0.08 ng/mL |

3. Normal range

Normal mouse serum, 400x diluted

Measured values were converted to actual mouse Neuropilin-1 concentration using dilution ratio.



4. Recovery test

Recombinant mouse Neuropilin-1 was added to samples at different concentration.

Serum 1 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 4.02 | – | – |
| 0.31 | 4.37 | 0.35 | 112% |
| 1.25 | 5.20 | 1.18 | 95% |
| 2.50 | 6.53 | 2.51 | 100% |

Serum 2 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 2.34 | – | – |
| 0.31 | 2.75 | 0.40 | 130% |
| 1.25 | 3.68 | 1.34 | 107% |
| 2.50 | 5.03 | 2.68 | 107% |

Serum 3 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 4.83 | – | – |
| 0.31 | 5.21 | 0.38 | 122% |
| 1.25 | 6.11 | 1.28 | 102% |
| 2.50 | 7.36 | 2.53 | 101% |

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技術資料や、関連情報はホームページからご利用頂けます。 <https://ruo.mbl.co.jp/product/cancer/abmatch.html>

■ 特徴

Ab-Match Assembly Mouse Neuropilin-1(CD304) kit は、Ab-Match Universal kit (MBL; code no. 5310) と組み合わせてご使用いただくことで、サンドイッチ ELISA 法にて試料中のマウス Neuropilin-1 を特異的に測定することができます。このセットで 96 穴マイクロプレート 1 枚分の測定が可能です。本製品は研究用試薬です。ヒトの体内に用いたり、診断の目的に使用しないでください。

■ キット構成 1 × 96 wells

| Name | Materials | Quantity | Dilution ratio |
|---|--|-----------------|----------------|
| Mouse Neuropilin-1 standard | Recombinant mouse Neuropilin-1* (lyophilized powder) | 1 vial | 1:10 – 1:640 |
| Anti-mouse Neuropilin-1 capture antibody | Rat anti-mouse Neuropilin-1 monoclonal antibody (50% Glycerol/PBS) | 60 μL × 1 vial | 1:200 |
| Anti-mouse Neuropilin-1 detection antibody | Biotin conjugated rat anti-mouse Neuropilin-1 monoclonal antibody | 110 μL × 1 vial | 1:101 |
| Streptavidin conj. peroxidase | Streptavidin conjugated peroxidase (SA-HRP) | 110 μL × 1 vial | 1:101 |

* Standardは蒸留水で溶解してください。溶解に用いる蒸留水の量はLotごとに異なりますので、"Mouse Neuropilin-1 standard" のチューブラベルの記載内容をご確認ください。

凍結融解を避けるため、溶解後は分注してください。

注) キットの構成は、室温 (20-30°C) に戻してから使用してください。

■ 操作法

| Coating step | Blocking step | Sample incubation step | Detection antibody incubation step | SA-HRP step | Substrate incubation step | Reading |
|---|---|--|--|---|--|---|
| 希釈した capture antibody を 100 μL ずつ各 well に分注 ↓ 2-8°C で一晩静置 | 2 回洗浄 ↓ Blocking Agent を 200 μL ずつ各 well に分注 ↓ 室温で 1 時間静置 | Well の内容物を完全に除去 ↓ 調整した standard 及びサンプルを 100 μL ずつ各 well に分注 ↓ 室温で 1 時間静置 | 4 回洗浄 ↓ 希釈した detection antibody を 100 μL ずつ各 well に分注 ↓ 室温で 1 時間静置 | 4 回洗浄 ↓ 希釈した SA-HRP を 100 μL ずつ各 well に分注 ↓ 室温で 30 分間静置 | 4 回洗浄 ↓ Substrate Solution を 100 μL ずつ各 well に分注 ↓ 室温で 30 分間静置 | Stop Solution を 100 μL ずつ各 well に分注 ↓ 450 nm にて吸光度を測定 |

■ 保存と有効

-20 ~ -30°C にて保存してください。有効期間は出荷後 12 ヶ月です。

■ 関連製品

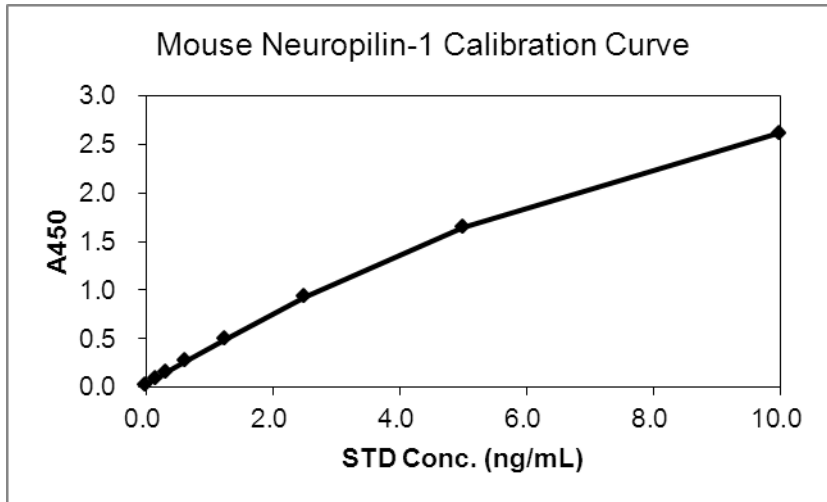
5310 Ab-Match Universal kit

■ 参考文献

- 1) Beck, B., *et al.*, *Nature* **478**, 399-403 (2011)
- 2) Bruder, D., *et al.*, *Eur. J. Immunol.* **34**, 623-630 (2004)
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■ 標準曲線例



■ 参考データ

1. 同時再現性

| ng/mL | CV % |
|-------|------|
| 0.94 | 3.2 |
| 2.96 | 1.4 |
| 3.85 | 2.4 |
| 5.84 | 3.1 |

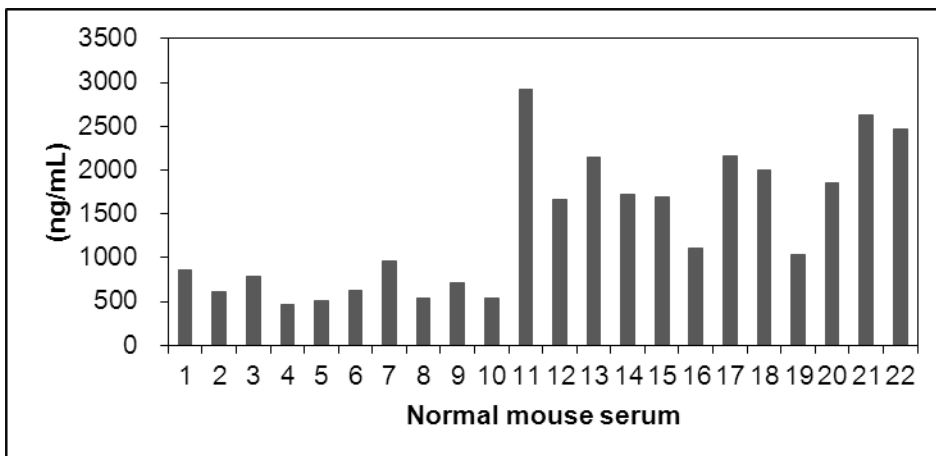
2. 検出感度

| | |
|--------------------------|-------------|
| LOB (Limit of Blank) | 0.051 ng/mL |
| LOD (Limit of Detection) | 0.08 ng/mL |

3. 基準範囲

正常マウス血清、400倍希釈を使用

測定値は、希釈倍率を基に実際のマウス Neuropilin-1 濃度に換算しております。



4. 添加回収試験

血清 3 例を用い、添加回収試験を行い、下表の結果を得ました。

Serum 1 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 4.02 | – | – |
| 0.31 | 4.37 | 0.35 | 112% |
| 1.25 | 5.20 | 1.18 | 95% |
| 2.50 | 6.53 | 2.51 | 100% |

Serum 2 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 2.34 | – | – |
| 0.31 | 2.75 | 0.40 | 130% |
| 1.25 | 3.68 | 1.34 | 107% |
| 2.50 | 5.03 | 2.68 | 107% |

Serum 3 (added to 400x diluted serum)

| (A) Added Mouse Neuropilin-1 (ng/mL) | Mouse Neuropilin-1 concentration measured (ng/mL) | (B) Recovery (ng/mL) | (B/A) Recovery (%) |
|--|---|-------------------------|-----------------------|
| 0.00 | 4.83 | – | – |
| 0.31 | 5.21 | 0.38 | 122% |
| 1.25 | 6.11 | 1.28 | 102% |
| 2.50 | 7.36 | 2.53 | 101% |