TGF-β1 (LAP) Rat Uncoated ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of rat TGF-β1 (LAP)

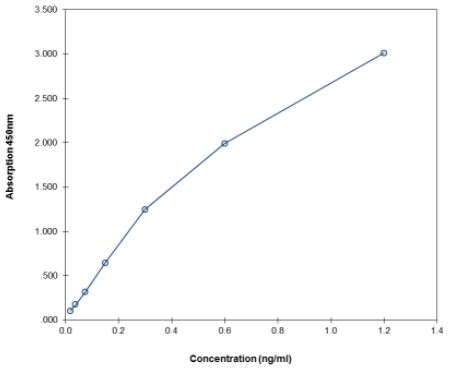
Catalog Number 88-50680

Pub. No. MAN0016766 Rev. B.0 (31)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Standard curve of TGF-β1 (LAP) Rat Uncoated ELISA Kit

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.



Product information

Symbol	Contents	TGF-β1 (LAP) Rat Uncoated ELISA Kit
REF	Catalog number	88–50680
—	Sensitivity	0.019 ng/ml
—	Standard curve range	0.019–1.20 ng/ml
	Temperature limitation	Store at 2–8°C
LOT	Batch code	Refer to vial
	Use by	Refer to box label
	Caution	Contains preservatives

Description

This TGF-β1 (LAP) Rat Uncoated ELISA Kit contains the necessary reagents, standards, buffers, and diluents for performing quantitative enzyme-linked immunosorbent assays (ELISA). This ELISA set is specifically engineered for accurate and precise measurement of rat TGF-β1 (LAP)protein levels from samples including serum, plasma, and supernatants from cell cultures.

Components of 2-plate format (2x96 tests)

- **Capture Antibody:** Pre-titrated, purified anti-rat TGF-β1 (LAP)monoclonal antibody

  1 vial (100 µL) Capture Antibody Concentrate (250X)
- **Detection Antibody:** Pre-titrated, biotin-conjugated anti-rat TGF-β1 (LAP) monoclonal antibody

  1 vial (50 µL) Detection Antibody Concentrate (250X)
- **Standard:** Recombinant rat TGF-β1 (LAP) for generating standard curve and calibrating samples

  2 vials rat TGF-β1 (LAP) Standard (lyophilized): 2.4 ng/ml upon reconstitution
- **Coating Buffer:** 1 vial (2.5 ml) Phosphate Buffered Saline Concentrate (PBS) 10X
- **Assay Buffer A:** 3 bottles (10 ml) Assay Buffer A Concentrate 20X (PBS with 1% Tween™-20 and 10% BSA)
- **Detection enzyme:** Pre-titrated Streptavidin-HRP

  1 vial (250 µL)
- **Substrate Solution:** Tetramethylbenzidine (TMB) Substrate Solution

  1 bottle (25 ml)
- **96-well plates:** 2 Corning™ Costar™ plates

Other materials needed

- Buffers
  - Wash Buffer: 1X PBS, 0.05% Tween™-20 or eBioscience™ Wash Buffer (20X) Cat. Nos. BMS408.0500 or 00-0400-46
  - Stop Solution: 1 M H_3PO_4 or 2 N H_2SO_4 or eBioscience™ Stop Solution Cat. Nos. BMS409.0100, SS03, SS03100, or SS04
- Pipettes and pipettors
- Refrigerator
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer

Note: To ensure optimal results from using this kit, use only the components included in the set. Exchanging of components is not recommended because a change in performance may occur.

Stability

This kit is guaranteed to perform as defined if stored and handled as instructed according to this datasheet and the Certificate of Analysis, which is included with the reagents. Expiration date is indicated on the box label.

Storage instructions for kit reagents

Store at 2-8°C.

Reagent preparation

Note: If crystals form in the buffer concentrate, warm them gently until they completely dissolve.

- **Coating Buffer (1X)**

  Make a 1:10 dilution of PBS (10X) in deionized water.
- **Blocking Buffer (2X)**

  Make a 1:10 dilution of Assay Buffer A Concentrate (20X) in deionized water.
- **Assay Buffer A (1X)**

  Make a 1:20 dilution of Assay Buffer A Concentrate (20X) in deionized water.
- **Capture Antibody**

  Dilute capture antibody (250X) 1:250 in Coating Buffer (1X).
- **Standard**

  Reconstitute rat TGF-β1 (LAP) standard by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Allow the standard to reconstitute for 10-30 minutes. Swirl or mix gently to ensure complete and homogeneous solubilization (concentration of reconstituted standard = 2.4 ng/ml).

  Mix well prior to making dilutions. The standard has to be used immediately after reconstitution and cannot be stored.
- **Detection Antibody**

  Dilute detection antibody (250X) 1:250 in Assay Buffer A (1X).
- **Streptavidin-HRP**

  Dilute Streptavidin-HRP 1:100 in Assay Buffer A (1X).

Experimental procedure

Note: In case of incubation without shaking, the obtained O.D. values may be decreased. Nevertheless the results are still valid.

Note: Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.

Note: If instructions of this protocol have been followed, samples have been diluted 1:500, the concentration read from the standard curve must be multiplied by the dilution factor (x500).

1. Coat Corning™ Costar™ 9018 ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted in Reagent preparation). Seal the plate and incubate overnight at 4°C.
2. Prepare the Blocking Buffer (see Reagent preparation).
3. Aspirate wells and wash twice with 400 µL/well Wash Buffer.

   Allowing time for soaking (~1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
4. Block wells with 250 µL of Blocking Buffer. Incubate at room temperature for 2 hours (or over night 4°C).
5. Prepare the Standard and Detection Antibody (see Reagent preparation).
6. Aspirate/wash as in step 3. Repeat for a total of two washes.
7. Perform 2-fold serial dilutions of the standards with Assay Buffer A (1X) to make the standard curve.

   For that add 100 µL of Assay Buffer A (1X) to all standard wells. Add 100 µL reconstituted standard in duplicate into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1=1.2 ng/ml) and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch surface of the microwells. Continue this procedure five times.
8. Add 100 µL/well of Assay Buffer A (1X) to the blank wells.
9. Add 80 µL/well of Assay Buffer A (1X) to the sample wells.
10. Add 20 µL/well of your prediluted samples to the appropriate wells, prediluting them at least 100-fold in Assay Buffer A (1x).
11. Add 50 µL/well diluted Detection Antibody to all wells.
12. Cover or seal the plate and incubate at room temperature for 2 hours **(Shaking is recommended).**
13. Prepare Streptavidin-HRP (see Reagent preparation).
14. Aspirate/wash as in step 3. Repeat for a total of four washes.
15. Add 100 µL/well diluted Streptavidin-HRP. Seal the plate and incubate at room temperature for 1 hour **(Shaking is recommended).**
16. Aspirate/wash as in step 3. Repeat for a total of four washes.
17. Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
18. Add 100 µL of Stop Solution to each well.
19. Read plate at 450 nm. If wavelength substraction is available, substract the values of 570 nm from those of 450 nm and analyze data.

ELISA troubleshooting guide

Problem	Possibility	Solution
High background	Improper and inefficient washing.	Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed.
	Cross contamination from other specimens or positive controls.	Repeat ELISA, be careful when washing and pipetting.
	Contaminated substrate.	Substrate should be colorless.
	Incorrect dilutions, e.g., conjugate concentration was too high.	Repeat test using correct dilutions; check with manufacturer.
No signal	Improper, low protein binding capacity plates were used.	Repeat ELISA, using recommended high binding capacity plates.
	Wrong substrate was used.	Repeat ELISA, use the correct substrate.
	Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity.	Repeat ELISA, make sure your system contains no enzyme inhibitor.
Very weak signal	Improper and inefficient washing.	Make sure washing procedure is done correctly.
	Incorrect dilutions of standard.	Follow recommendations of standard handling exactly as written on the certificate of analysis.
	Insufficient incubation time.	Repeat ELISA, follow the protocol carefully for each step's incubation time.
	Incorrect storage of reagents.	Store reagents in the correct temperature, avoid freeze and thaw, avoid using the frost free freezer.
	Wrong filter in ELISA reader was used.	Use correct wavelength setting.
	Wrong plate used.	Use the recommended Corning™ Costar™ 9018 or Nunc™ MaxiSorp™ flat bottom 96-well plates.
Variation among replicates	Improper and inefficient washing.	Make sure washing procedure is done correctly; see certificate of analysis.
	Poor mixing of samples.	Mix samples and reagents gently and equilibrate to proper temperature.
	Plates not clean.	Plates should be wiped on bottom before measuring absorbance.
	Improper, low binding capacity plates were used.	Use recommended high binding capacity plates.
	Reagents have expired.	Do not use if past expiration date.
Variation of kit performance	Different buffers, plates. Handling can strongly affect kit performance.	Use eBioscience™ buffers, plates, and kit components available.

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
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