

RESEARCH MONOGRAPH · COMPOUND PROFILE

TB-500

A 43-residue acetylated synthetic peptide derived from the actin-sequestering protein thymosin beta-4, studied for tissue repair, cell migration, and angiogenic endpoints.

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ABSTRACT

TB-500 is the common research-grade designation for a synthetic acetylated peptide whose primary sequence is a fragment of human thymosin beta-4 (Tbeta4), a 43-residue actin-binding protein expressed at high concentration in platelets, leukocytes, and many other cells. The most frequently cited research-grade preparation is the full-length Tbeta4 sequence (43 amino acids; molecular weight approximately 4963 Da; CAS 77591-33-4) acetylated at the N-terminus to mimic the native post-translational modification. A small minority of vendor literature has used "TB-500" to refer to a shorter active fragment (LKKTETQ, residues 17 through 23 of Tbeta4), which is a different molecule with a different mass; investigators should confirm by mass spectrometry which sequence has been supplied. This monograph documents the full-length acetylated Tbeta4 sequence supplied as the Kodiak biolabs lot of record. Reported activities span actin sequestration, fibroblast and endothelial cell migration, angiogenic remodeling, attenuated inflammatory cytokine response, and accelerated repair of cardiac, dermal, corneal, and tendon injury models. Routes studied in the preclinical literature include intravenous, intramuscular, subcutaneous, intraperitoneal, oral, and topical administration. Plasma half-life following parenteral administration is short, on the order of one hour, but tissue retention extends substantially longer. Two Phase 2 trials of recombinant Tbeta4 in dry eye and pressure ulcer indications have been reported with mixed efficacy outcomes. The compound is not approved by any regulatory authority for human or veterinary use. The principal limitations on the strength of the published evidence are the variation in

commercial preparations sold as "TB-500" and the scarcity of large independent randomized human trials.

1. Compound identification

1.1. Nomenclature and synonyms

The most common research-grade designation is "TB-500." The full-length parent molecule is thymosin beta-4 (Tbeta4, TMSB4X gene product). Less common synonyms in the literature include T beta 4, TB4, and the developmental compound code RGN-352 used by RegeneRx Biopharmaceuticals during clinical development. The CAS number for the full-length Tbeta4 sequence is 77591-33-4. The PubChem CID is 16131459. PMID searches return more than 4,000 records under the Tbeta4 root term; the "TB-500" string is largely confined to research-grade vendor literature and a smaller body of preclinical work.

The persistent ambiguity in the research-grade market is whether "TB-500" refers to the full 43-mer Tbeta4 sequence or to the shorter active fragment LKKTETQ (residues 17-23). The fragment retains many of the biologically relevant actin-sequestering and migratory effects in published assays but has a molecular weight of approximately 889 Da rather than 4963 Da. The two preparations are not interchangeable. The lot of record at Kodiak biolabs (lot 20260224) is the full-length sequence as confirmed by mass spectrometry on the vendor analytical report.

1.2. Structural chemistry

The full-length Tbeta4 sequence is N-acetyl-Ser-Asp-Lys-Pro-Asp-Met-Ala-Glu-Ile-Glu-Lys-Phe-Asp-Lys-Ser-Lys-Leu-Lys-Lys-Thr-Glu-Thr-Gln-Glu-Lys-Asn-Pro-Leu-Pro-Ser-Lys-Glu-Thr-Ile-Glu-Gln-Glu-Lys-Gln-Ala-Gly-Glu-Ser. The peptide carries a high density of charged residues (six lysines, eight glutamic acids, three aspartic acids) and a single methionine, with no cysteines or aromatic residues other than two phenylalanines and one tyrosine in the parent protein. The N-terminal acetylation is biologically important; deacetylated synthetic preparations show reduced activity in actin-binding assays. The molecular formula is approximately C₂₁₂H₃₅₀N₅₆O₇₈S; the average molecular weight is 4963.45 g/mol. The lot of record carries a vendor-reported HPLC purity of 99.87 percent.

In aqueous solution Tbeta4 exists predominantly as a random-coil, intrinsically disordered peptide. The actin-binding interaction induces a transient amphipathic alpha-helical fold over the central WLKKTETQ stretch (positions 17-23) when the peptide engages the hydrophobic cleft of monomeric G-actin. The central LKKTETQ epitope is the basis on which the shorter "TB-500 fragment" preparations were developed; the surrounding residues are required for the full conformational rearrangement.

1.3. Physicochemical properties

The full-length Tbeta4 peptide is supplied as a white to off-white lyophilized powder with high solubility in water, bacteriostatic water, 0.9 percent saline, and standard biological buffers. The lot of record (Kodiac lot 20260224) conforms to this description. Solubility in pure DMSO is poor; DMSO is not the appropriate vehicle. Lyophilized peptide stored at minus 20 degrees Celsius in the original sealed vial is stable for at least 24 months under standard light protection. Reconstituted solutions in bacteriostatic water are stable for at least 30 days at 2 to 8 degrees Celsius; some published preparations have demonstrated stability up to 60 days under refrigeration. Freeze-thaw cycles should be avoided; aliquoting is the standard practice for working solutions.

The isoelectric point is approximately 4.6 due to the excess of acidic residues. At physiological pH the peptide carries a substantial net negative charge. The peptide does not form aggregates at the concentrations used in standard reconstitution protocols (1 to 5 mg/mL) and is fully soluble at 10 mg/mL in bacteriostatic water for short-term use.

2. Discovery and developmental history

2.1. The thymosin family

Thymosin beta-4 was first isolated from calf thymus extracts in 1981 by Teresa Low and Allan Goldstein at the George Washington University, building on the earlier characterization of the thymosin family of immunoregulatory factors. The cDNA was sequenced shortly thereafter and revealed a small acidic peptide with broad tissue distribution. By the late 1990s a coherent picture had emerged: Tbeta4 is the dominant intracellular G-actin-sequestering peptide in most cell types, with intracellular concentrations in the hundreds of micromolar range, particularly in platelets and leukocytes.

The functional shift from "intracellular actin sequestrant" to "extracellular signaling peptide" emerged in a separate line of work that documented circulating Tbeta4 in plasma at low nanomolar concentrations and demonstrated extracellular effects on cell migration, angiogenesis, and inflammation. The release mechanism remains incompletely characterized; both controlled secretion and platelet-degranulation-dependent release have been documented.

2.2. Clinical translation

RegeneRx Biopharmaceuticals (now G-treatment Inc.) advanced recombinant full-length Tbeta4 (developmental name RGN-352) through Phase 1 and Phase 2 trials in the early 2000s and 2010s. A Phase 2 trial in chronic neurotrophic dry eye disease reported improved corneal epithelial healing and reduced ocular discomfort scores. A Phase 2 trial in stage III pressure ulcer reported faster wound closure relative to placebo. A separate program evaluated topical Tbeta4 in epidermolysis bullosa with positive preliminary results. None of these programs reached Phase 3 or regulatory approval; commercial development stalled in part for funding reasons rather than efficacy concerns.

There is no FDA-approved IND for "TB-500" as a separate compound; the regulatory record reflects work on recombinant full-length Tbeta4 under the developmental name RGN-352. The compound is not a regulated drug substance in the

United States, the European Union, or Japan. Clinical use in humans, where it occurs, is off-label and outside the scope of regulatory oversight; this monograph does not endorse such use.

3. Mechanism of action

3.1. Actin sequestration

The primary biochemical mechanism is sequestration of monomeric G-actin. Tbeta4 binds G-actin with a dissociation constant of approximately 0.4 to 1 micromolar, occupying the hydrophobic cleft of the actin monomer and preventing nucleation and polymerization until the actin is released to F-actin polymers under conditions of cytoskeletal remodeling. The intracellular pool of G-actin available for rapid polymerization is buffered by Tbeta4; depletion or sequestration of Tbeta4 alters the kinetics of actin-dependent processes including cell migration, phagocytosis, and platelet shape change.

3.2. Cell migration and wound healing

The migratory effects on fibroblasts, endothelial cells, and keratinocytes are documented across multiple wound-healing models. The mechanism is not solely a passive consequence of actin sequestration; Tbeta4 also activates the integrin-linked kinase (ILK) and AKT signaling pathways, upregulates laminin-5 expression in keratinocytes, and modulates the expression of beta-catenin in tissue repair contexts. The functional consequence is accelerated re-epithelialization of dermal and corneal wounds and accelerated reorganization of granulation tissue.

3.3. Angiogenesis

Tbeta4 promotes endothelial cell migration, tube formation in vitro, and capillary formation in vivo. The mechanism involves both direct effects on endothelial actin remodeling and indirect effects through upregulation of angiogenic growth factors including VEGF and bFGF. In ischemic injury models, Tbeta4 administration accelerates microvascular reorganization at the injury site and reduces ischemic damage volume.

3.4. Inflammation modulation

Tbeta4 attenuates pro-inflammatory cytokine release in models of acute injury and chronic inflammation. Reported effects include reduced TNF-alpha and IL-6 production by activated macrophages, reduced NF-kappa-B activation in endothelial cells exposed to inflammatory stimuli, and attenuated neutrophil infiltration in tissue injury models. The anti-inflammatory mechanism appears to be partially independent of the actin-sequestering activity and may involve direct interaction with the gelsolin-actin-binding pathway and modulation of cellular oxidant production.

3.5. Cardiac repair

In the most cited preclinical line of work, intracardiac or systemic Tbeta4 administration following experimental myocardial infarction reduces infarct size, accelerates cardiomyocyte survival in the peri-infarct zone, and promotes recruitment of epicardial progenitor cells to the injury site. The mechanism involves both anti-apoptotic signaling in mature cardiomyocytes and reactivation of an embryonic cardiogenic program in the adult epicardium. Subsequent independent replication has been mixed; some groups reproduce the protective effect, others find smaller or null effects depending on dose, route, and timing.

3.6. Limits of the mechanistic model

The mechanistic literature is rich but heterogeneous. Effects on actin sequestration are firmly established at the biochemical level. Effects on cell migration, angiogenesis, and inflammation are documented across multiple laboratories but with variable effect sizes that depend strongly on the model, the dose, and the timing of administration. Cardiac protection is replicated in some labs and not in others. Investigators should treat the mechanistic claims as supported but with effect sizes that may be smaller than the most-cited preclinical reports suggest.

4. Pharmacokinetics

4.1. Routes of administration

Published preclinical work includes intravenous, intramuscular, subcutaneous, intraperitoneal, oral, intratracheal, intracardiac, intracerebral, ophthalmic, and topical administration. Subcutaneous and intramuscular administration are the most common parenteral routes in research-grade dosing and produce predictable absorption with peak plasma concentrations within 30 to 60 minutes of injection. Oral bioavailability is poor; the peptide is degraded substantially in gastric acid and serum peptidases before reaching systemic circulation, with reported oral bioavailability below 5 percent in most published studies. Topical application is effective for dermal and corneal wound healing endpoints because the peptide is taken up locally without requiring systemic distribution.

4.2. Plasma half-life and tissue distribution

Plasma half-life following intravenous administration in rodents is approximately 60 to 90 minutes. Tissue retention is substantially longer; injected radiolabeled Tbeta4 is detectable in skeletal muscle, dermis, and granulation tissue for up to 72 hours after a single dose, consistent with accumulation in extracellular matrix and slow release. The tissue half-life relevant for repair endpoints is therefore on the order of days, not minutes, despite the short plasma half-life.

4.3. Metabolism and excretion

Tbeta4 is hydrolyzed by serum and tissue peptidases at multiple sites. The major metabolites include the LKKTETQ fragment (positions 17-23), which retains some biological activity, and shorter inactive fragments including the tetrapeptide AcSDKP (N-acetyl-seryl-aspartyl-lysyl-proline), an antifibrotic peptide that contributes to some of the parent compound's reported effects in cardiac and renal fibrosis models. Renal excretion of the parent peptide and its fragments is the primary clearance route. The compound is not a known inducer or inhibitor of major CYP isoforms at expected exposure ranges.

5. Reported research dose ranges

The published preclinical literature reports rodent doses ranging from 100 micrograms per kilogram to 12 milligrams per kilogram by various routes, with the majority of efficacy studies clustering around 500 micrograms to 6 milligrams per kilogram subcutaneously, two to three times per week. Allometric scaling to human equivalent doses (dividing by approximately 6 to 7 to account for body surface area) places the equivalent human exposure in the range of 70 micrograms to 1 milligram per kilogram per administration, or approximately 5 to 70 milligrams per dose for a 70 kg subject.

The dose range commonly cited in research-grade vendor literature (2 to 5 milligrams per research subject, twice per week for the first 4 to 6 weeks, then a maintenance dose of 2 to 5 milligrams once weekly) is broadly consistent with the lower end of the allometrically scaled efficacy range. The Phase 2 ophthalmic and dermal trials used topical preparations at concentrations of 0.01 to 0.1 percent and parenteral systemic doses up to 10 milligrams per dose. Dose-response data in the published literature are sparse and do not extend cleanly to humans; investigators should treat published research-grade dose ranges as approximate guidance rather than validated dosing.

6. Sourcing and quality verification

6.1. Synthesis route

TB-500 is produced by solid-phase peptide synthesis using Fmoc chemistry. The 43-residue length is at the upper bound of routine SPPS without segment condensation, which makes coupling efficiency the critical bottleneck. The most common synthetic problems are deletion sequences (one or more residues skipped during coupling, producing 42-mer or 41-mer impurities of similar HPLC retention), incomplete N-terminal acetylation (producing the unacetylated free-amine form, which has reduced activity), and racemization at sensitive residues during prolonged synthesis at elevated temperature.

The lot of record (Kodiak biolabs lot 20260224) was synthesized by the supplier of record and shipped in lyophilized acetate salt form. The vendor analytical report records 99.87 percent purity by HPLC and confirms identity by mass spectrometry. The COA report omitted an explicit HPLC field; the purity value is sourced from the accompanying HPLC report supplied with the lot.

6.2. Common adulterants and impurities

The research-grade TB-500 supply chain has documented patterns of substandard product. Reported issues include:

1. Substitution of the shorter LKKTETQ fragment (approximately 889 Da) for the full 43-mer (approximately 4963 Da) without disclosure. The two compounds are not equivalent and have different dose-response profiles; mass spectrometry on every batch is the only reliable check.
2. Unacetylated N-terminus, producing a peptide of correct length but reduced activity. The free amine adds about 42 daltons relative to the N-acetyl form; mass spectrometry resolves this readily.
3. Deletion sequences from incomplete coupling, primarily at the prolines and at the central LKKTETQ stretch. These fragments are inactive and inflate apparent purity by HPLC if they co-elute with the parent peak.

4. Excess acetate or trifluoroacetate counterions inflating apparent mass by 5 to 12 percent depending on salt form.
5. Endotoxin contamination from non-pyrogen-controlled synthesis, particularly relevant for in vivo work.

Independent verification by HPLC and mass spectrometry on every batch is essential. The vendor COA is necessary but not sufficient; a rigorous sourcing model includes a third-party lab. Kodiak biolabs is in the process of establishing this third-party verification through Janoshik Analytical; as of this revision only the vendor analytical report is available, and that limitation is acknowledged on every product page.

6.3. Analytical standards

Reverse-phase HPLC on a C18 column with gradient elution from aqueous TFA (0.1 percent) to acetonitrile, monitoring at 215 nm and 280 nm, is the standard purity assay. Identity confirmation by LC-MS with electrospray ionization yields a series of multiply charged ions; the $[M+5H]^{5+}$ peak at approximately 993.7 m/z is convenient for quantification, with the full deconvoluted molecular ion at 4963 Da. The acceptance criteria applied by the vendor lab and replicated in the planned Janoshik verification are: total purity above 98.0 percent at both wavelengths, identity confirmation within 0.5 ppm of theoretical mass, and absence of major impurity peaks above 0.5 percent of the parent peak.

7. Reconstitution and handling

7.1. Solvent system

TB-500 dissolves readily in water, bacteriostatic water, and 0.9 percent saline. The standard reconstitution protocol uses bacteriostatic water (0.9 percent benzyl alcohol preservative) for any solution intended to be stored beyond a single use. For acute in vitro experiments where preservative is undesired, sterile water for injection or PBS works equally well. DMSO is not the appropriate vehicle; the peptide does not require organic co-solvent and DMSO at concentrations above approximately 5 percent introduces vehicle effects in cellular assays.

7.2. Reconstitution protocol

Add the calculated volume of solvent slowly along the side wall of the vial. A 5 mg vial reconstituted in 2 mL produces a 2.5 mg/mL solution. Allow the vial to rest at room temperature for 60 to 90 seconds, then gently swirl until the powder fully dissolves. The peptide is highly soluble; full dissolution is typically complete within 90 seconds. Visual inspection should confirm a clear solution without particulates or turbidity. The peptide is not vortex-sensitive but vigorous shaking introduces foaming and is unnecessary.

7.3. Storage stability

Lyophilized TB-500 in the unopened vial: stable at minus 20 degrees Celsius for at least 24 months under the conditions documented in the lot expiration date. Reconstituted solutions: stable at 2 to 8 degrees Celsius for at least 30 days, with extended stability up to 60 days under controlled refrigeration. Aliquot working solutions to avoid freeze-thaw cycles. Reconstituted solutions should not be stored at room temperature beyond 24 hours.

8. Stack interaction considerations

Combination with BPC-157 is the most commonly cited stack in research-grade literature, on the rationale that BPC-157 contributes vascular and matrix effects while TB-500 contributes actin-binding and migratory effects. The mechanistic case for synergy is plausible but not formally documented in controlled comparison studies; investigators should treat the stack as additive rather than synergistic absent specific experimental evidence in their model of interest.

Combination with growth hormone secretagogues (Ipamorelin, CJC-1295) is reported in research-grade literature on the basis that GH-axis stimulation may potentiate TB-500's effects on connective tissue repair. The mechanistic case is plausible because GH receptor signaling and IGF-1 axis activity intersect with several Tbeta4-modulated pathways, but no controlled comparison data support a specific synergy claim.

Pharmacokinetic interactions with CYP-substrate compounds are not expected at standard research dose ranges due to the peptide's minimal hepatic metabolism. There is no published evidence of receptor-level interactions with conventional small-molecule pharmacotherapy.

9. References

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