Development of Sandwich Immunoassays for Quantification of Total TGF-β1, Free Active TGF-β1, Latency-Associated Peptide (LAP), Latent TGF-β, and Soluble GARP in Biological Samples

04-0040-00

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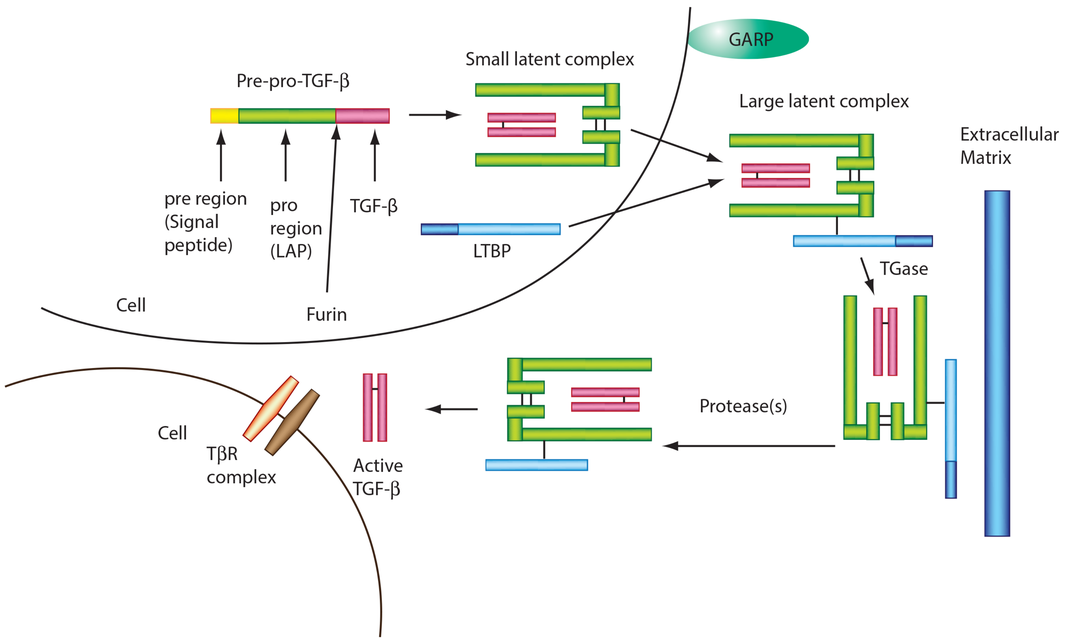
Abstract

TGF-β controls cell proliferation and differentiation and many other biological processes. As a circulating protein, TGF-β1 exists predominantly in a complex form with latency-associated peptide (LAP), forming small latent TGF-β , and subsequently with latent TGF-β-binding protein (LTBP), forming large latent TGF-β, which further interacts with other proteins in circulation. TGF-β1 also exists in a free active homodimeric form that plays direct biological roles. The cell surface receptor for small latent TGF-β is GARP, which may also exist in a soluble form. The complex nature of the system necessitates analytical methods to quantify these components individually in order to understand their exact roles. Here, we describe development of individual immunoassays to quantify total TGF-β1, free active TGF-β1, LAP, latent TGF-β, and soluble GARP. The antibodies and recombinant protein standards used in these assays were developed and manufactured at BioLegend. The sandwich assays were analytically and biologically validated to ensure that assay methods are specific, sensitive, accurate, reproducible, and robust. The assays were tested with cell culture supernatant, serum, and plasma samples. The free active TGF-β1 assay allows detection of low pg/mL concentrations in sample, which were not previously possible. Assays for LAP, latent TGF-β, and soluble GARP provide novel tools to study their roles in regulating TGF-β activities and their own biological functions. Now commercially available, these assay products are valuable tools for biomedical studies.

Introduction



MATERIALS AND METHODS

- All assays are sandwich ELISAs.
- Antibodies: Monoclonal antibodies were produced at BioLegend. A polyclonal antibody used as a detection antibody for soluble GARP was purchased commercially.
- Protein Standards: Recombinant TGF-β1 was produced at BioLegend. Recombinant LAP and GARP were purchased commercially. Culture supernatant from latent TGF-β transfectant was developed internally at BioLegend and was used as latent TGF-β standard.
- All buffers, substrate solutions, avidin-HRP, and other reagents were produced at BioLegend.

CONCLUSIONS

- TGF-β1, Latency-associated peptide, Latent TGF-β and GARP play important roles in many biological processes. No good commercial reagents were available for quantification of active TGF-β1, latent TGF-β and soluble GARP.
- We successfully developed ELISA kits for quantification of total TGF-β1, free active TGF-β1, LAP, latent TGF-β, and soluble GARP for biological samples.
- The free active TGF-β1 kit has high sensitivity (low pg/mL) and does not require sample treatment.
- The latent TGF-β1 and soluble GARP ELISAs are the first commercial products available.
- The assays are analytically validated for robustness (specificity, accuracy, sensitivity, reproducibility, stability, biological relevance, etc.) for use in biomedical research.
- We also developed ELISA products for mouse and rat TGF-β1 (total and free active), and mouse latent TGF-β.

Figure 1. Standard Curves for Human Total TGF-β1, Free Active TGF-β1, LAP, Latent TGF-β, and Soluble GARP ELISA Assays

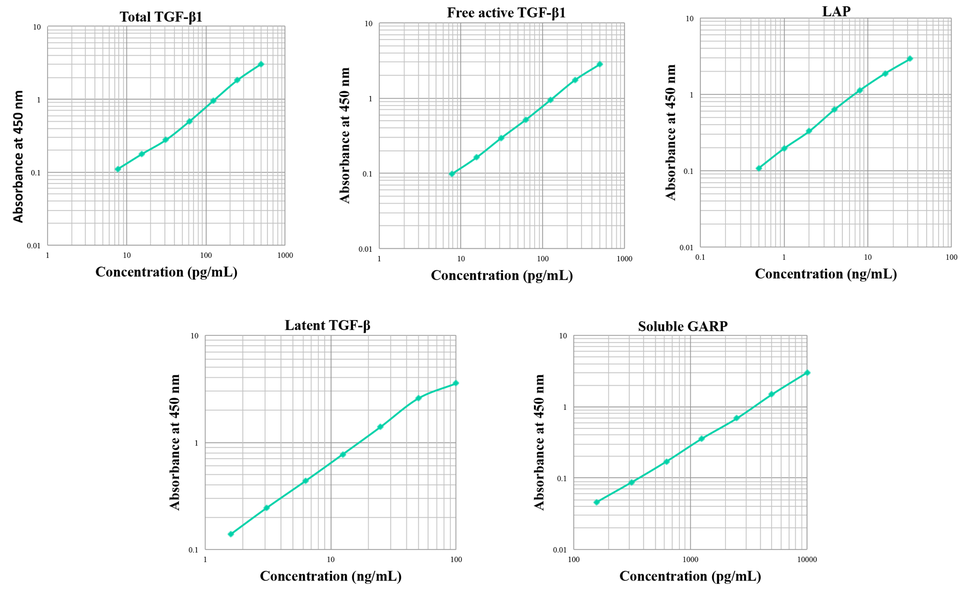


Table 1. Major Analytical Characteristics of the ELISA Products for Quantification of Human TGF-β1, Free Active TGF-β1, LAP, Latent TGF-β, and Soluble GARP

Parameters	Total TGF-β1	Free Active TGF-β1	LAP	Latent TGF-β	Soluble GARP
Specificity	No cross reactivity with 87 human cytokines. 0.1% cross reactivity with LAP	No or negligible cross reactivity with LAP and 87 human cytokines.	No cross reactivity with TGF-β1 and 70 different human cytokines. ~13% cross reactivity with human latent TGF-β	No or negligible cross reactivity with TGF-β1, LAP, and 18 human cytokines	No cross reactivity with TGF-β1, LAP, latent TGF-β, and 23 human cytokines
Sensitivity (MinDC)	1.62 ± 0.3 pg/mL	2.3 ± 0.3 pg/mL	0.167 ± 0.05 ng/mL	0.153 ± 0.04 ng/mL	51.5 ± 5.1 pg/mL
Assay Accuracy (Serum)	76%	76%	100%	99%	85%
Serum Linearity of Dilution	98% (1:100, 1:200)	95% (1:2, 1:4, 1:8, 1:16)	101% (1:20, 1:40, 1:80)	106% (1:20, 1:40, 1:80)	107% (1:2, 1:4, 1:8)
Intra-assay Precision (CV)	4% (N = 16)	6.5% (N = 16)	7% (N = 16)	5.1% (N = 16)	1.2% (N = 16)
Inter-assay Precision (CV)	6.5% (N = 7)	7.0% (N = 7)	9% (N = 4)	3.6% (N = 4)	4.8% (N = 6)
Serum Sample Dilution	1:100	Neat undiluted	1:10	1:10	Neat undiluted
Sample Volume required	5 µL	50 µL	5 µL	5 µL	50 µL
Assay Performance Stability	Stable	Stable	Stable	Stable	Stable
Sample Freeze/Thaw Stability	Stable	Stable	Stable	Stable	Stable
Sample Treatment	Acidification/ Neutralization	N/A	N/A	N/A	N/A
Assay Time (Hours)	<4.0	<4.0	<4.0	<4.0	5.0

Table 2. Typical Concentrations for Total TGF-β1, Free Active TGF-β1, LAP, Latent TGF-β, and Soluble GARP in Human Serum Samples

Serum ID	TGFβ1 (ng/mL)	LAP (ng/mL)	Latent TGFβ (ng/mL)	Free Active TGF-β1* (pg/mL)	Soluble GARP* (pg/mL)
1	2.43	0.90	34.0	30.0	129.0
2	1.75	0.90	22.0	92.0	ND
3	2.85	0.70	33.0	302.0	108.0
4	3.42	73.50	117.0	102.0	316.0
5	4.31	4.80	42.0	37.0	136.0
6	3.26	1.70	39.0	40.0	ND
7	2.93	5.00	29.0	60.0	124.0
8	1.75	1.80	27.0	83.0	318.0
9	1.32	6.00	16.0	26.0	113.0
10	2.26	0.10	26.0	19.0	ND
Average	2.63	9.54	38.5	79.1	124.0
Median	2.64	1.75	31.0	50.0	118.0
N	10	10	10	10	10

*Free active TGF-β1 and Soluble GARP were assayed with different sets of human sera.