

Fig. 1



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Dulbecco's modified eagle medium (DMEM)

Product use

Dulbecco's modified eagle medium was developed in 1969 and is a modification of basal medium eagle (BME) that differs from BME and MEM by the following characteristics:

- Vitamins 4X greater than MEM. Vitamins and amino acids greater than BME
- Types and quantities of amino acids greater than MEM and BME
- Iron (ferric nitrate)

Description

- 12-604 With 4.5 g/L glucose, with L-glutamine
 12-614 With 4.5 g/L glucose, without L-glutamine
 12-707 With 1.0 g/L glucose, without L-glutamine
 12-708 With 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
 12-709 With 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine
 12-733 With 4.5 g/L glucose, without L-glutamine, without sodium pyruvate
 12-741 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate
 12-914 With 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth
 12-917 With 4.5 g/L glucose, without L-glutamine or phenol red
 15-604 With 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder
 15-614 With 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder
 (Powder formulations require the addition of 49.3 mL of NaHCO₃ 7.5% solution or 3.70 g/L of NaHCO₃ powder)

Quick reference chart

	Glucose	L-glutamine	phenol red	HEPES buffer	Sodium pyruvate
12-604	4.5 g/L	+	+	-	+
12-614	4.5 g/L	-	+	-	+
12-707	1.0 g/L	-	+	-	+
12-708	1.0 g/L	-	+	+	+
12-709	4.5 g/L	-	+	+	+
12-733	4.5 g/L	-	+	-	-
12-741	4.5 g/L	+	+	-	-
12-914	4.5 g/L	-	+	-	+
12-917	4.5 g/L	-	-	-	+

Fig. 1 continued



Specifications

	Sterility	pH	Osmolality (mOsm)	Cell growth promotion (% of control)	Endotoxin (EU/ml)
12-604	Neg.	7.0-7.4	324-352	≥75%	FIO
12-614	Neg.	7.0-7.4	324-352	≥75%	FIO
12-707	Neg.	7.0-7.4	306-346	≥75%	FIO
12-708	Neg.	7.03-7.27	300-326	≥75%	FIO
12-709	Neg.	7.0-7.4	321-351	≥75%	FIO
12-733	Neg.	7.0-7.4	318-360	≥75%	FIO
12-741	Neg.	7.0-7.4	318-360	≥75%	FIO
12-914	Neg.	7.0-7.4	324-352	≥85% **	FIO
12-917	Neg.	7.0-7.4	329-343	≥75%	FIO
15-604 ***	-	5.5-7.0	235-265	≥75%	≤1.0
15-614 ***	-	5.5-7.0	235-265	≥75%	≤1.0

FIO – for information only

** Result of functional test

*** Moisture content #1.0

Storage

2°C to 8°C

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

Catalog number	Description	Size
12-604F	DMEM with 4.5 g/L glucose, with L-glutamine	500 ml
12-604Q	DMEM with 4.5 g/L glucose, with L-glutamine	1 L
12-614F	DMEM with 4.5 g/L glucose, without L-glutamine	500 ml
12-614Q	DMEM with 4.5 g/L glucose, without L-glutamine	1 L
12-707F	DMEM with 1.0 g/L glucose, without L-glutamine	500 ml
12-708F	DMEM with 1.0 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-709F	DMEM with 4.5 g/L glucose, and 25 mM HEPES buffer, without L-glutamine	500 ml
12-733F	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	500 ml
12-733Q	DMEM with 4.5 g/L glucose, without L-glutamine, without sodium pyruvate	1 L
12-741F	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate	500 ml
12-914F	DMEM with 4.5 g/L glucose, without L-glutamine, screened to support hybridoma growth.	500 ml
12-917F	DMEM with 4.5 g/L glucose, without L-glutamine or phenol red.	500 ml
15-604D	DMEM with 4.5 g/L glucose, with L-glutamine, without sodium pyruvate, powder	1 x 10L
15-604F	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 X 50L
15-614D	DMEM with 4.5 g/L glucose, without L-glutamine or sodium pyruvate, powder	1 x 10L

Fig. 2



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Ham's F12 Medium

Product Use

Ham's F12 Medium is a nutrient mixture designed to cultivate a wide variety of mammalian and hybridoma cells when used with serum in combination with hormones and transferrin.

Specifications

Sterility	pH	Osmolality (mOsm)
Negative	7.07-7.40	286-305
Cell Growth Generation	Endotoxin (EU/ml)	
≥75% of control	FIO	

Storage

2°C to 8°C

Product Use Statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering Information

Catalog Number	Description	Size
12-615F	Ham's F12 Medium with L-glutamine	500 ml

Fig. 3



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DMEM: F12 (1:1) medium

Product use

DMEM combined with Hams F12 has been extensively used to demonstrate the effect of various hormones and growth factors on target tissues.

Description

- 12-719 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose
- 15-719 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of NaHCO₃, 7.5% solution or 1.2 g/L NaHCO₃ powder
- 04-687 with 3.151 g/L glucose, with L-glutamine, without HEPES

Specifications

	Sterility	pH	Osmolality (mOsm)
12-719	Negative	7.0-7.4	286-356
15-719*	Negative	4.5-6.5	260-290
04-687	Negative	FIO	FIO
	Cell growth promotion	Endotoxin (EU/ml)	Moisture
12-719	≥ 75% of control	FIO	--
15-719*	≥ 75% of control	≤ 1 EU/ml	≤ 2%
04-687	--	FIO	--

* pH, osmolality, endotoxin, and moisture are done without NaHCO₃; NaHCO₃ is added for cell growth test.

Storage

2°C to 8°C

Product use statement

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

Ordering information

Catalog number	Description	Size
12-719F	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	500 ml
12-719Q	DMEM: F12 with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose	1 L
15-719D	DMEM: F12 powder – with L-glutamine, 15 mM HEPES, and 3.151 g/L glucose. Powdered medium requires the addition of 16.0 ml/L of NaHCO ₃ , 7.5% solution or 1.2 g/L NaHCO ₃ powder	1 x 10L
04-687Q	DMEM: F12 with 3.151 g/L glucose, with L-glutamine, without HEPES	1 L

Fig. 4

Cascade Biologics™
invitrogen cell culture



Medium 171, Medium 171PRF, and MEGS

Medium 171

M-171-500

500 ml

Medium 171PRF (Phenol Red-Free)

M-171PRF-500

500 ml

Product Description

Medium 171 and Medium 171PRF are sterile, liquid tissue culture media intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Medium 171 is a basal medium containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. Medium 171PRF is a phenol red-free version of Medium 171. These media do not contain antibiotics, antimycotics, hormones, growth factors, or proteins. These media are HEPES and bicarbonate buffered and are designed for use in an incubator with an atmosphere of 5% CO₂/95% air. To support plating and long-term proliferation of normal human mammary epithelial cells, these media must be supplemented with Mammary Epithelial Growth Supplement (MEGS, cat. no S-015-5).

Intended Use

Medium 171 is intended for use in the routine culture of normal human mammary epithelial cells. Medium 171PRF is intended for use by investigators who wish to culture normal human mammary epithelial cells in the absence of phenol red. When supplemented with MEGS, these media will support the plating and proliferation of normal human mammary epithelial cells at densities between 2.5×10^3 cells/cm² and 8×10^4 cells/cm². **This product is for research use only. Not for use in animals, humans, or diagnostic procedures.**

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

Medium 171 and Medium 171PRF are stored at 4° C in our facility and are shipped at ambient temperature. Upon receipt, these media should be stored at 4° C and should not be frozen. **Protect from light.** Several components of these tissue culture media are light-labile, and we recommend that the media not be exposed to light for lengthy periods of time. If the media are warmed prior to use, do not exceed 37° C. When stored in the dark at 4° C, the product is stable until the expiration date on the label.

Preparation of Supplemented Medium 171

1. Thaw one bottle of MEGS. Take one bottle of Medium from cold storage. Make sure that the caps of the vessels are tight.
2. Gently swirl the bottle of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
3. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
4. Using sterile technique in a laminar flow culture hood, transfer the entire contents of the bottle of supplement to the bottle of Medium.
5. Tightly cap the bottle of supplemented medium and swirl the contents to ensure a homogeneous solution. Avoid causing the medium to foam.

Storage and Stability of Supplemented Medium 171

Once Medium 171 or Medium 171PRF has been supplemented with MEGS, the supplemented medium should be stored in the dark at 4° C and should not be frozen. When stored in the dark at 4° C, the supplemented medium is stable for 1 month.

Selected References

The Medium 171 formulation is based on medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984

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MAN0001585

Page 1 of 2

Revised: 30 May 2009

Fig. 4 continued

Cascade Biologics™
invitrogen cell culture



MEGS Mammary Epithelial Growth Supplement

Cat. no. S-015-5
5 ml

Product Description

Mammary Epithelial Growth Supplement (MEGS) is a sterile, concentrated (100X) solution intended for use as one component in a complete culture environment for the growth of normal human mammary epithelial cells. Each 5 ml bottle of MEGS contains all of the growth factors, hormones, and tissue extracts necessary for the culture of normal human mammary epithelial cells and is the correct amount of supplement for a 500 ml bottle of Medium 171 or Medium 171PRF. MEGS is an ionically-balanced supplement containing bovine pituitary extract (BPE), bovine insulin, hydrocortisone and recombinant human epidermal growth factor. When a 500 ml bottle of Medium 171 or Medium 171PRF is supplemented with MEGS, the final concentrations of the components in the supplemented medium are: BPE, 0.4% v/v; bovine insulin, 5 µg/ml; hydrocortisone, 0.5 µg/ml; and recombinant human epidermal growth factor, 3 ng/ml.

Intended Use

MEGS is intended for use in conjunction with Medium 171 or Medium 171PRF for the routine serum-free culture of normal human mammary epithelial cells. ***This product is for research use only. Not for use in animals, humans, or diagnostic procedures.***

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Limited Use Label License No. 5: Invitrogen Technology

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Page 2 of 2

Revised: 30 May 2009

Storage and Stability

MEGS is stored at -20° C at our facility and is shipped on dry ice. Upon receipt, the product should be stored at -20° C in a freezer that is not self-defrosting. When stored at -20° C, the product is stable until the expiration date shown on the label.

After long-term storage at -20° C, MEGS may contain a small amount of precipitate. This precipitate is formed from cold-insoluble material in the BPE component of the MEGS and will not affect the performance of the product.

Thawing

To thaw, place the product in a 37° C water bath or overnight at 4° C. If thawed in a water bath, do not leave the product at 37° C after the product has thawed. For instructions on adding MEGS to Medium 171, please refer to the instructions that accompany the basal medium.

Selected References

The MEGS formulation is based on published supplementation of medium MCDB 170, with modifications.

Hammond SL, Ham RG, Stampfer MR; PNAS 81:5435-5439, 1984.

Fig. 5**PTT6 Medium ingredients list**

Medium composition List	Company Name	Catalogue Number
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Basic media

DMEM (also referred to herein as PTT6-basal medium)	Lonza	12-604F
DMEM/F12	Lonza	12-719F
M171	Life technologies	M171500

Serum

Fetal Bovine Serum	GE Healthcare	A15-151
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Antibiotic

Penicillin-Streptomycin-Amphotericin B	Lonza	17-745E
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Supplements

Adenine (optional)	Sigma	A8626-25G
Hydrocortisone (optional)	Sigma	H-0888
Epidermal Growth Factor	Millipore	GF-144
T3 (3,3',5-Triiodo-L-thyronine sodium salt) (optional)	Sigma	200-223-5
Recombinant Human Insulin AOF	Life Technologies	A11382IJ

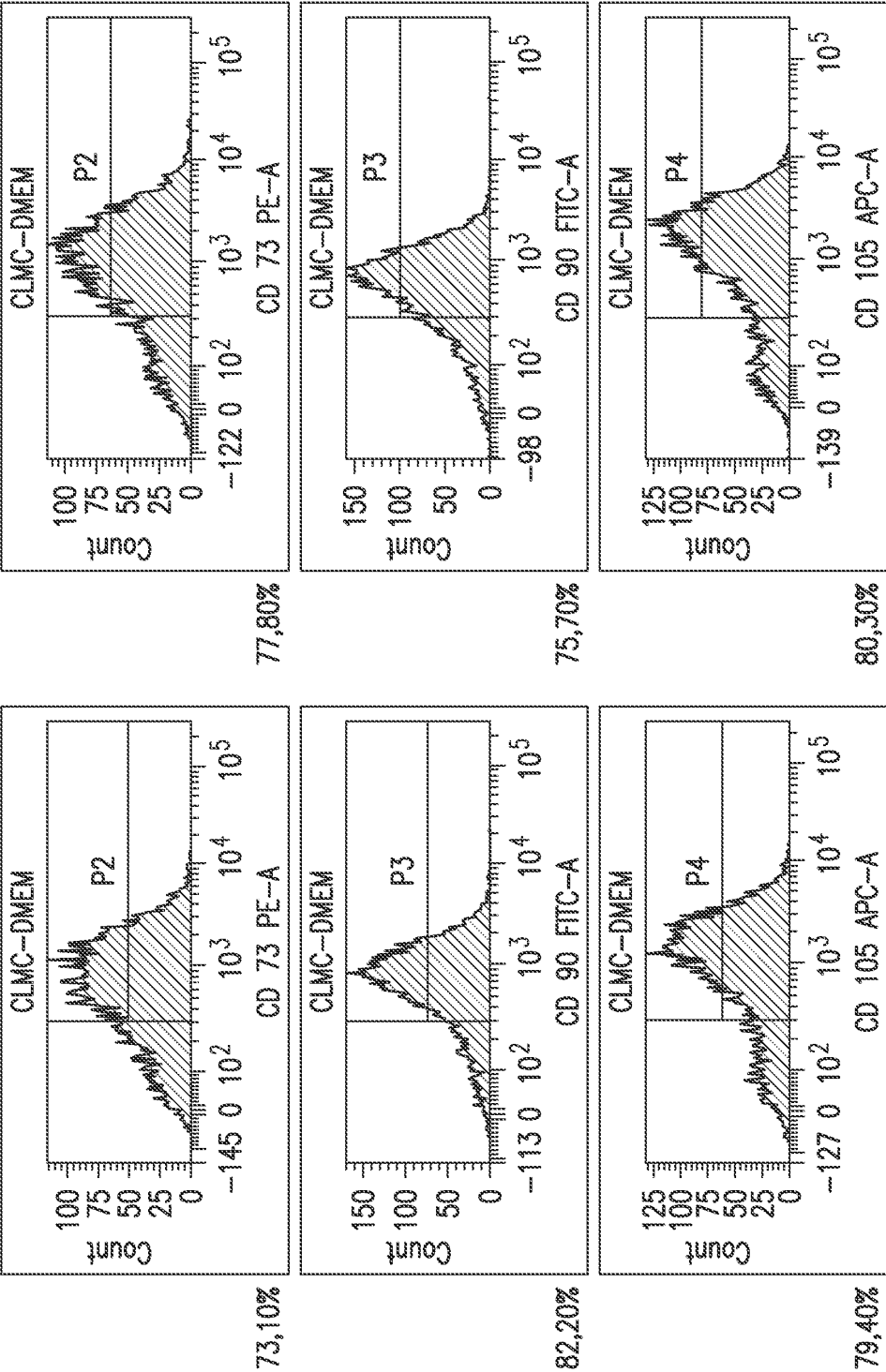


FIG.6A

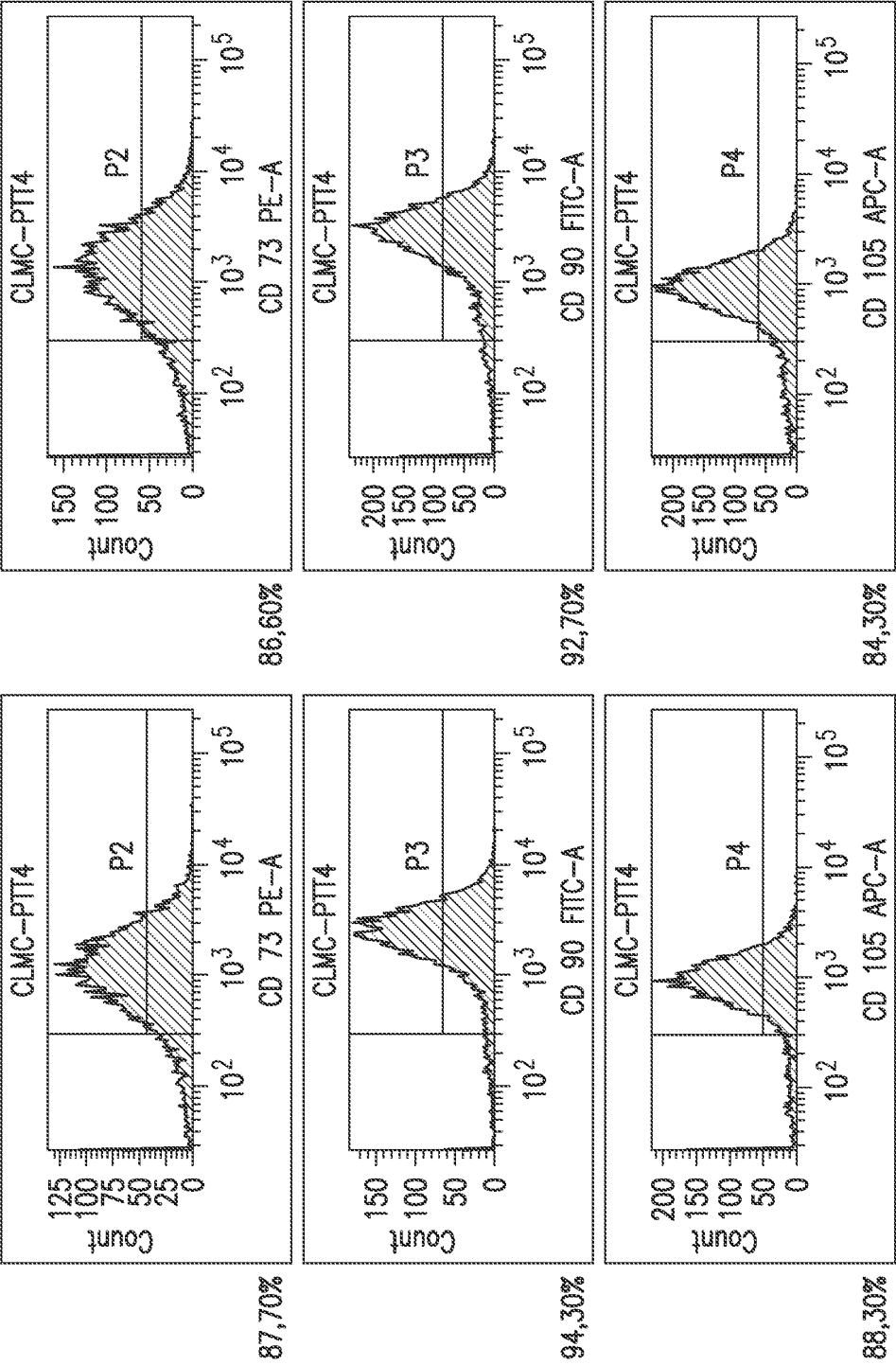


FIG.6B

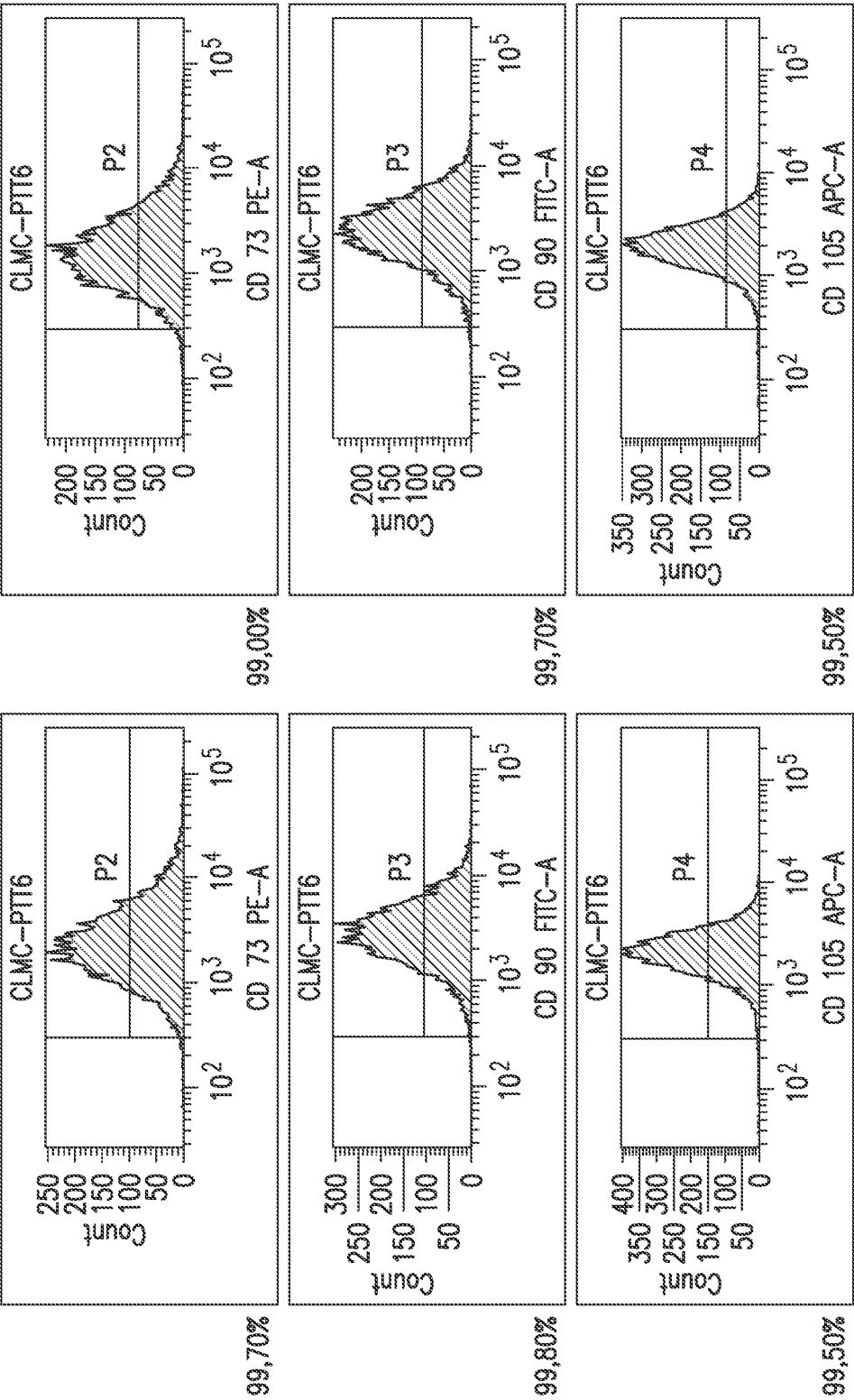
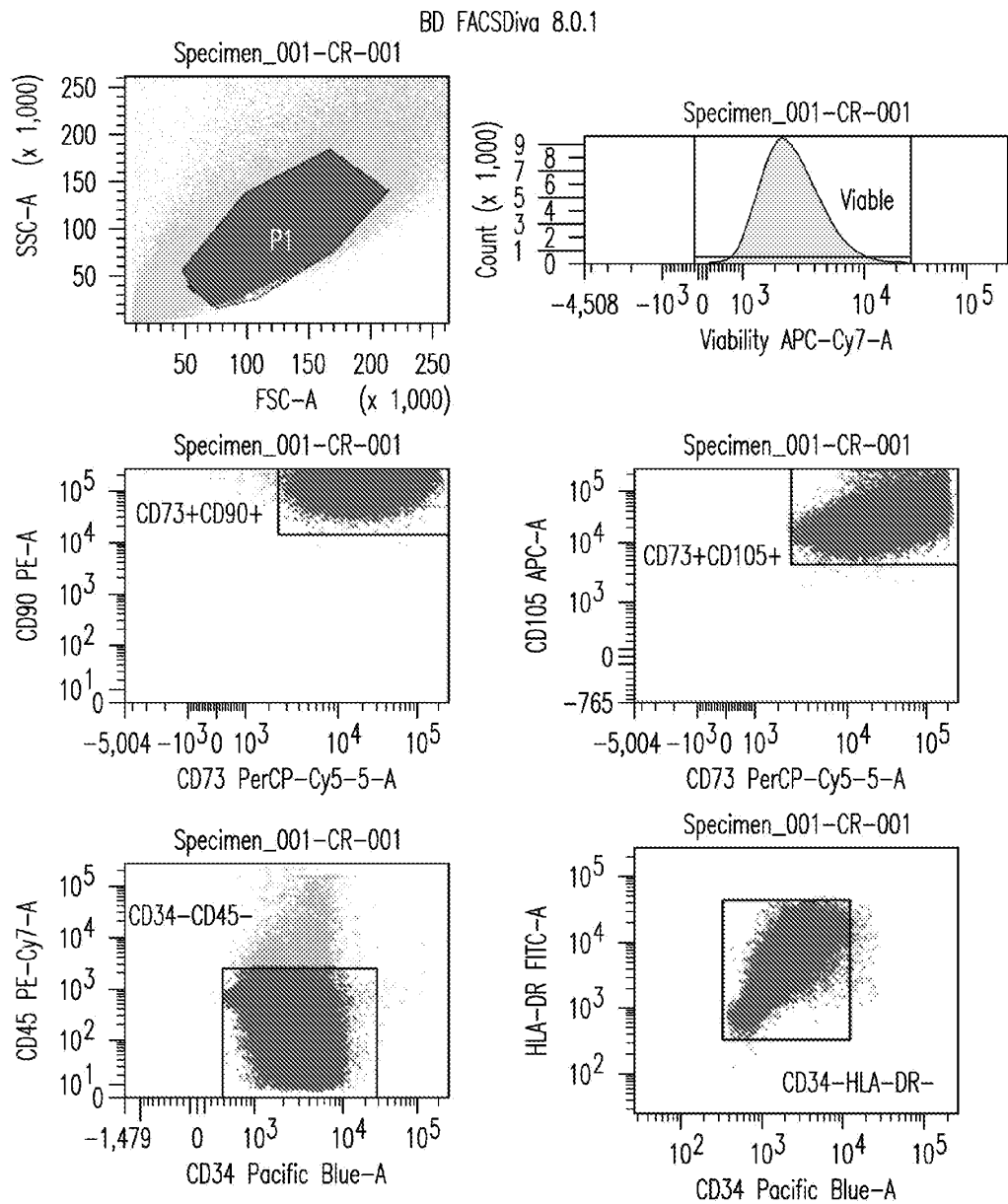
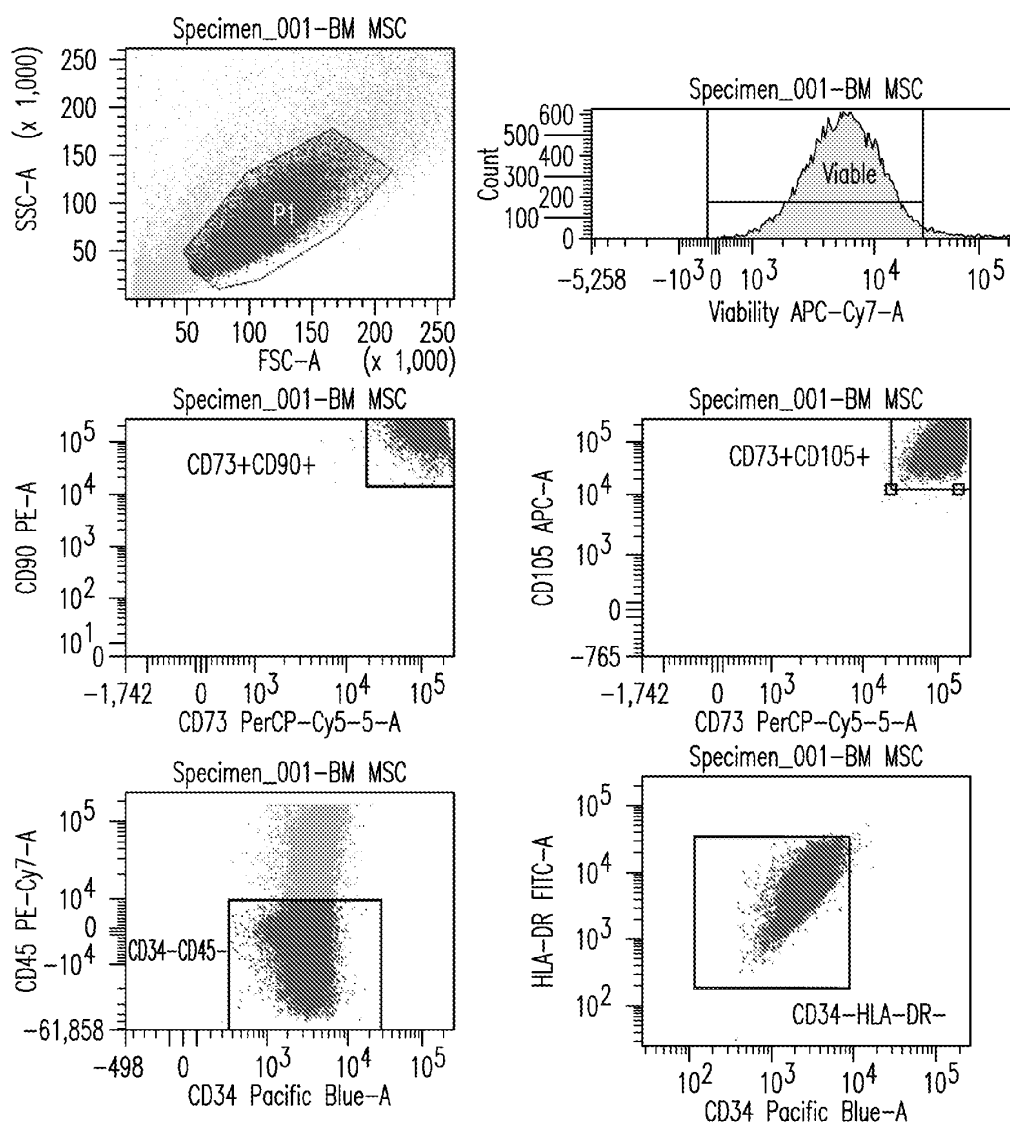


FIG.6C



Tube: CR-001			
Population	#Events	%Parent	%Total
All Events	540.988	####	100.0
P1	425.093	78.6	78.6
Viabile	414.643	97.5	76.6
CD73+CD90+	414.554	100.0	76.6
CD73+CD105+	414.490	100.0	76.6
CD34-CD45-	411.206	99.2	76.0
CD34-HLA-DR	411.061	100.0	76.0

FIG.7A



Experiment Name:	MSC 062216		
Specimen Name:	Specimen_001		
Tube Name:	BM MSC		
Population	#Events	%Parent	
CD34-HLA-DR-	21.128	99.9	
Population	#Events	%Parent	%Total
All Events	77.022	###	100.0
P1	35.780	46.5	46.5
Viable	33.741	94.3	43.8
CD73+CD90+	33.729	100.0	43.8
CD73+CD105+	33.710	99.9	43.8
CD34-CD45-	21.155	62.8	27.5
CD34-HLA-DR	21.128	99.9	27.4

FIG.7B

Figure 8

	1	2	3	4	5	6	7	8	9	10	11	12
A		Std. 1		CL-MSC in PTT4				Std. 1		CL-MSC in PTT6		
B		Std. 2		WJ-MSC in PTT4				Std. 2		WJ-MSC in PTT6		
C		Std. 3		AT-MSC in PTT4				Std. 3		AT-MSC in PTT6		
D		Std. 4		BM-MSC in PTT4				Std. 4		BM-MSC in PTT6		
E		Std. 5		CL-MSC in PTT4				Std. 5		CR001A		
F		Std. 6		WJ-MSC in PTT4				Std. 6		CR001C		
G		Std. 7		AT-MSC in PTT4				Std. 7		CR001D		
H		Blank		BM-MSC in PTT4				Blank		CR001G		

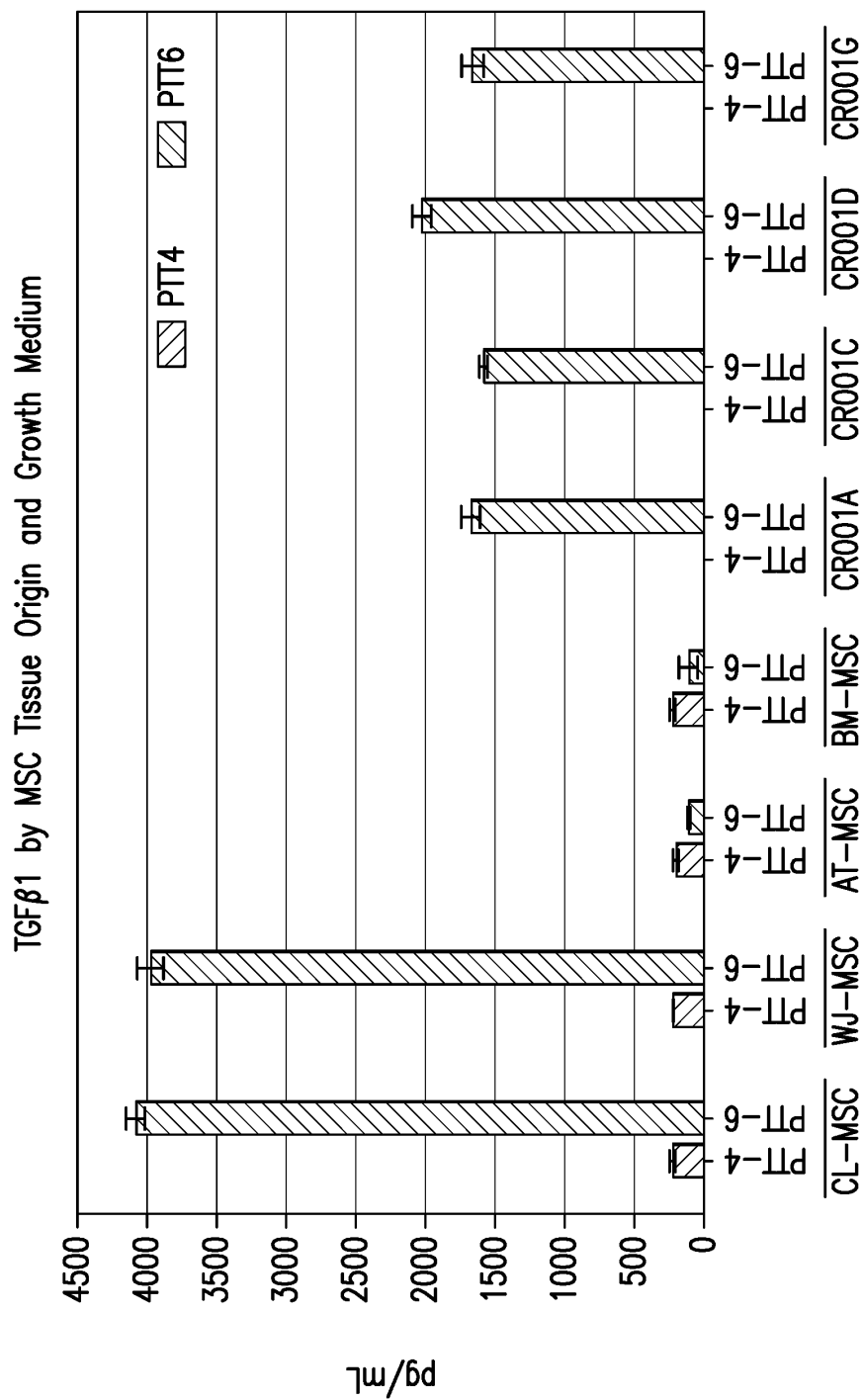


FIG.9

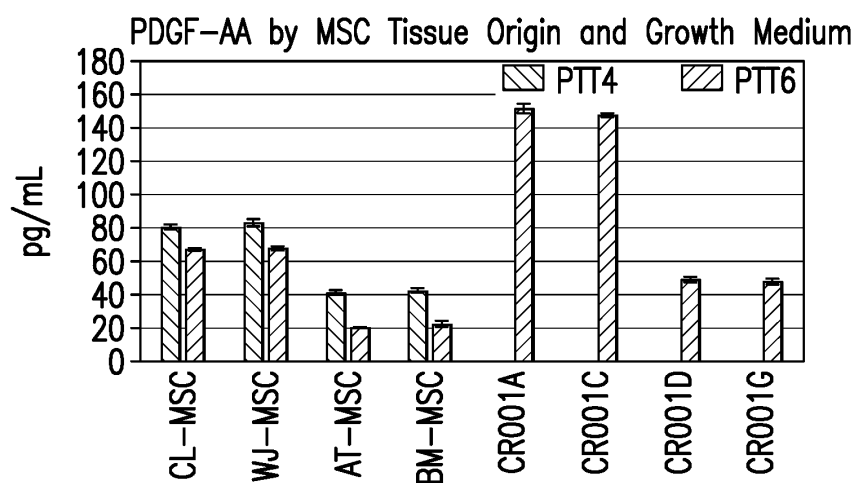


FIG.10A

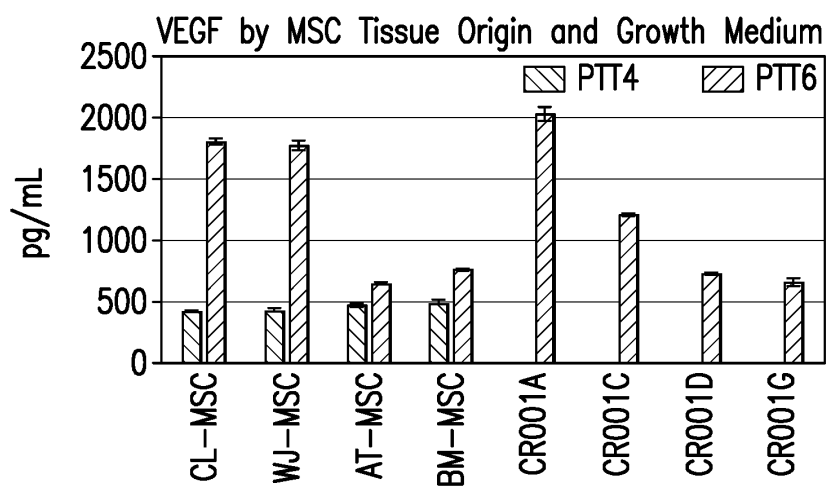


FIG.10B

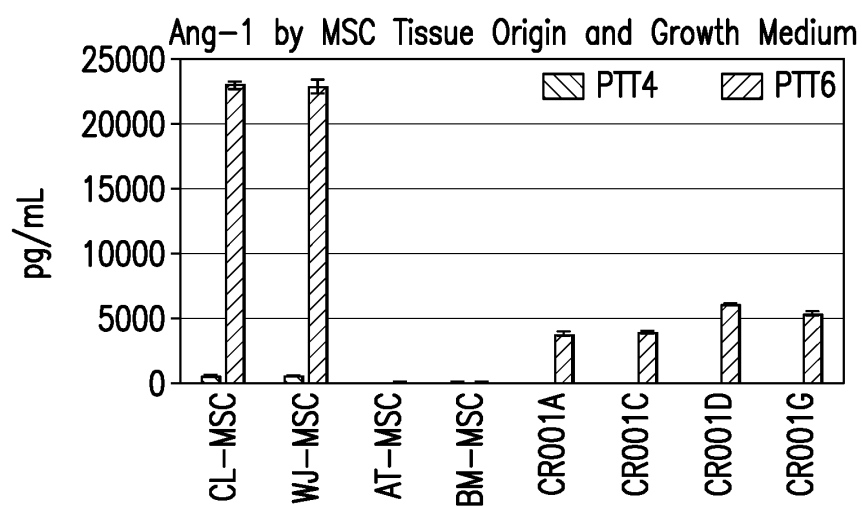


FIG.10C

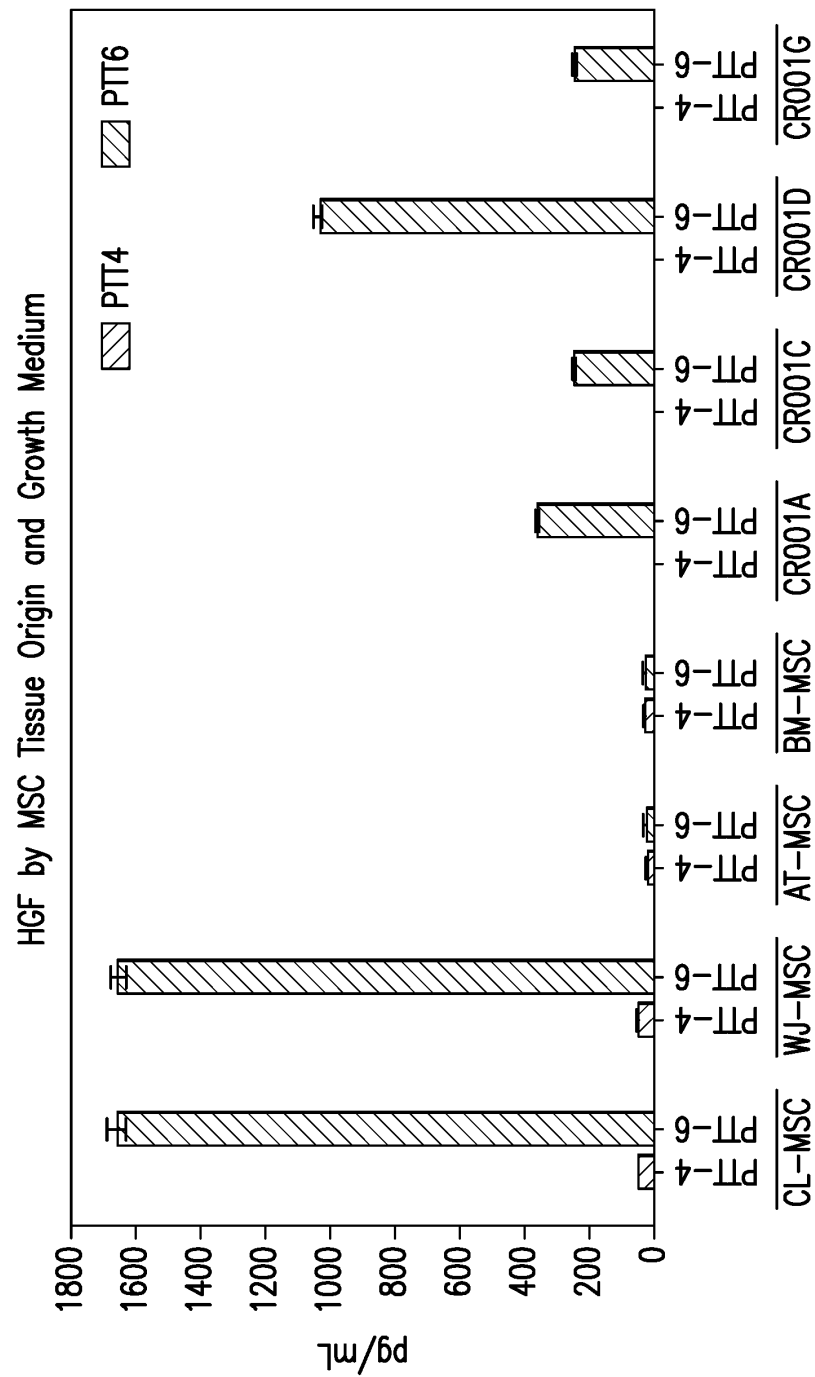


FIG.11

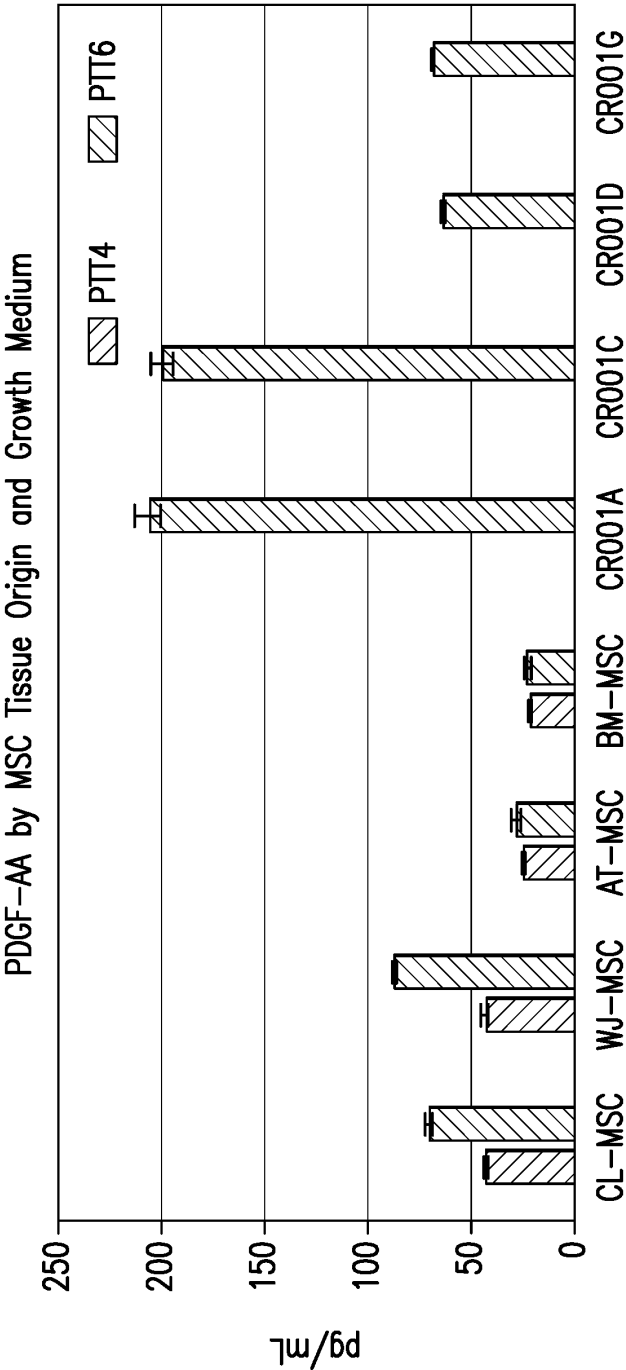


FIG.12

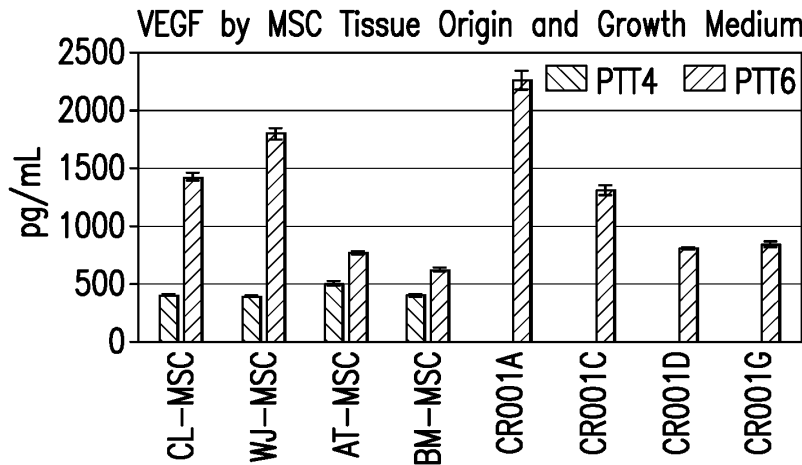


FIG.13A

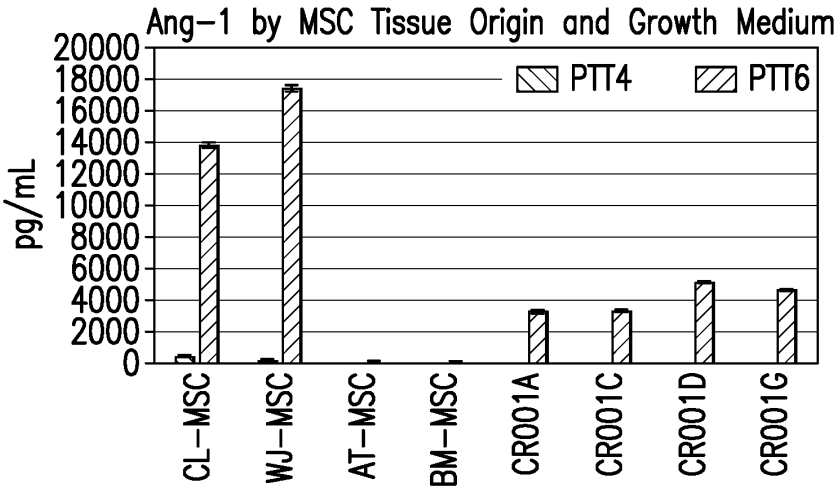


FIG.13B

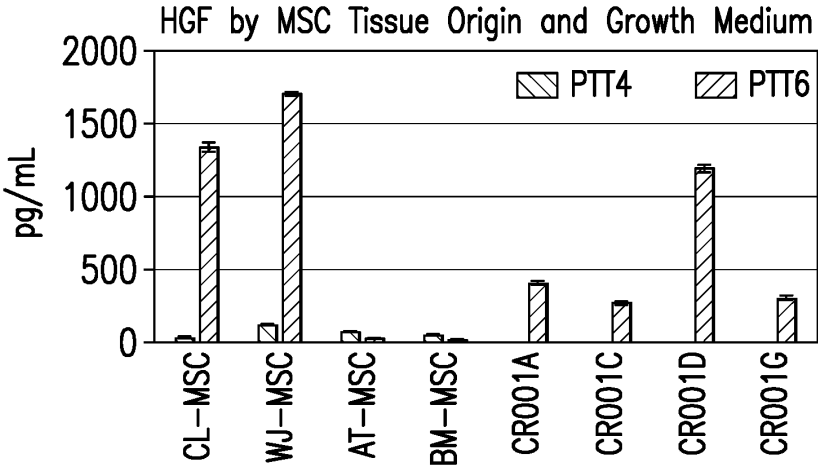


FIG.13C

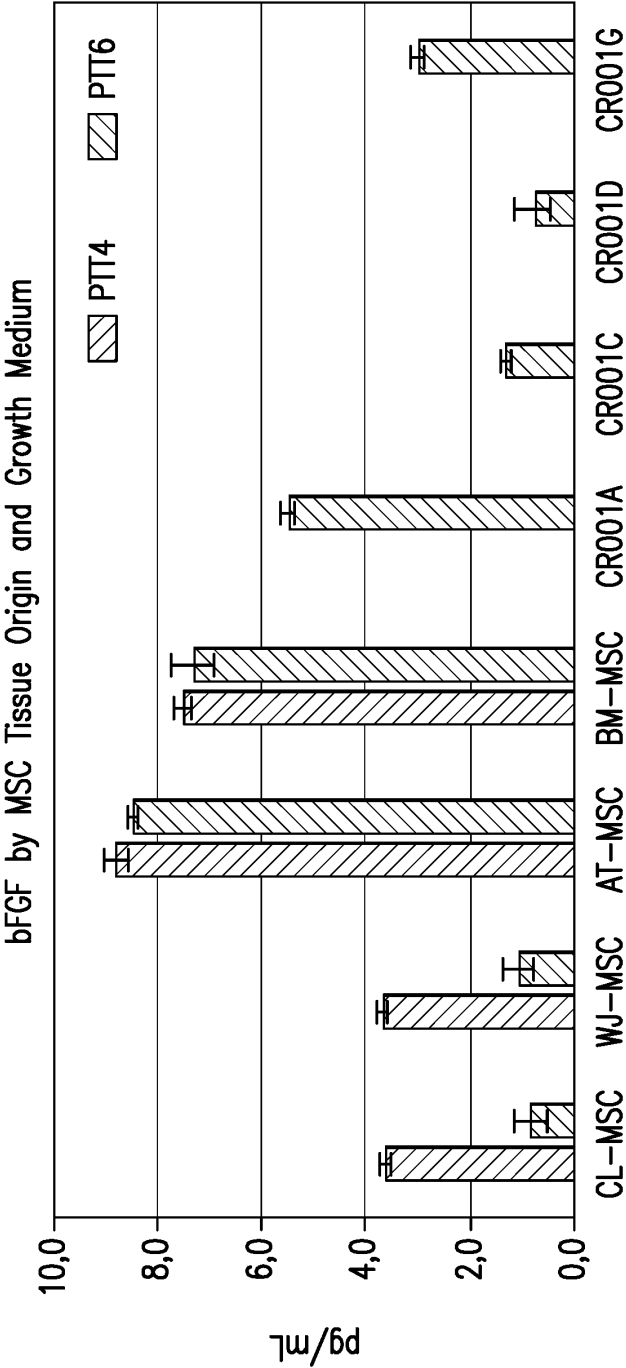


FIG.14

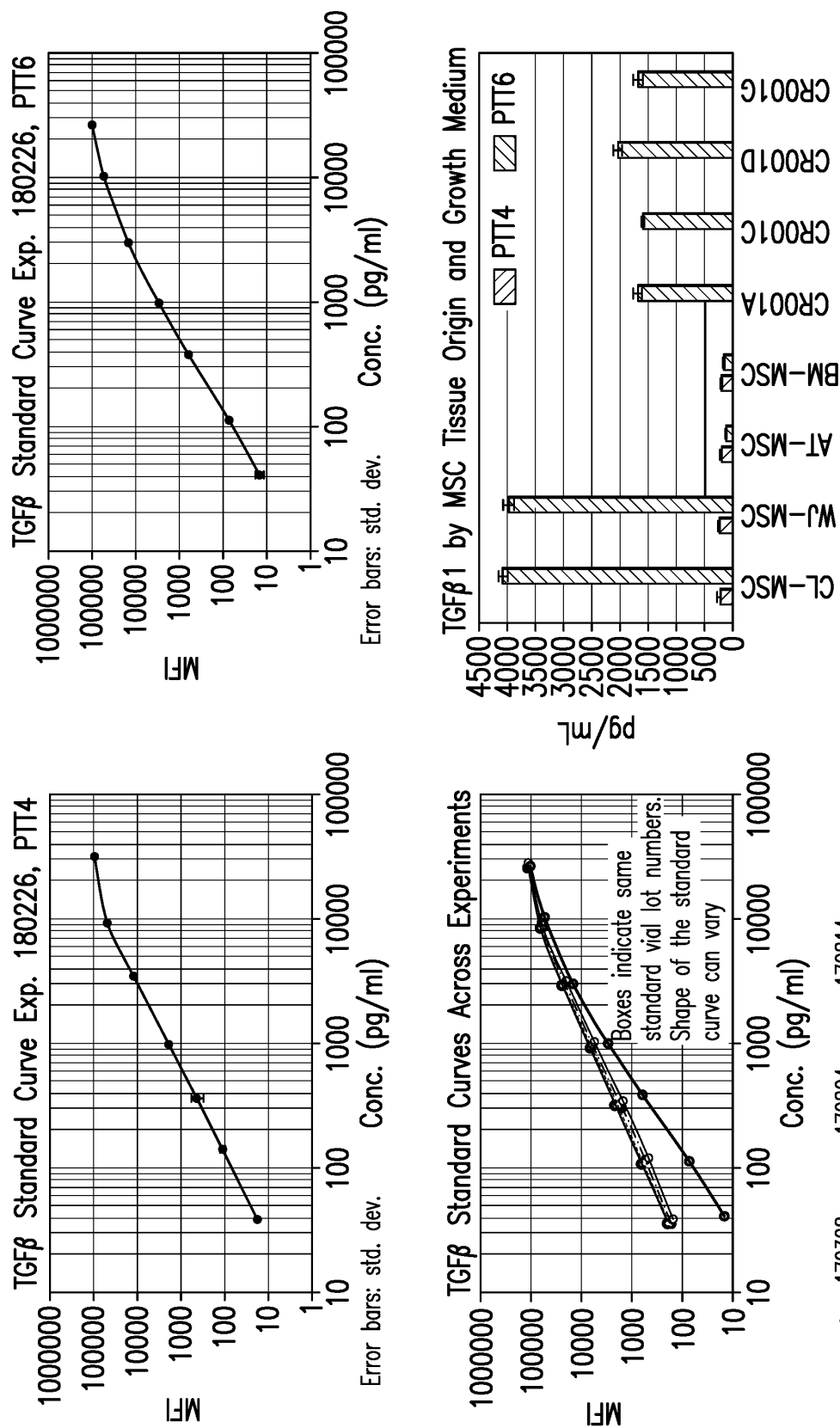


FIG.15

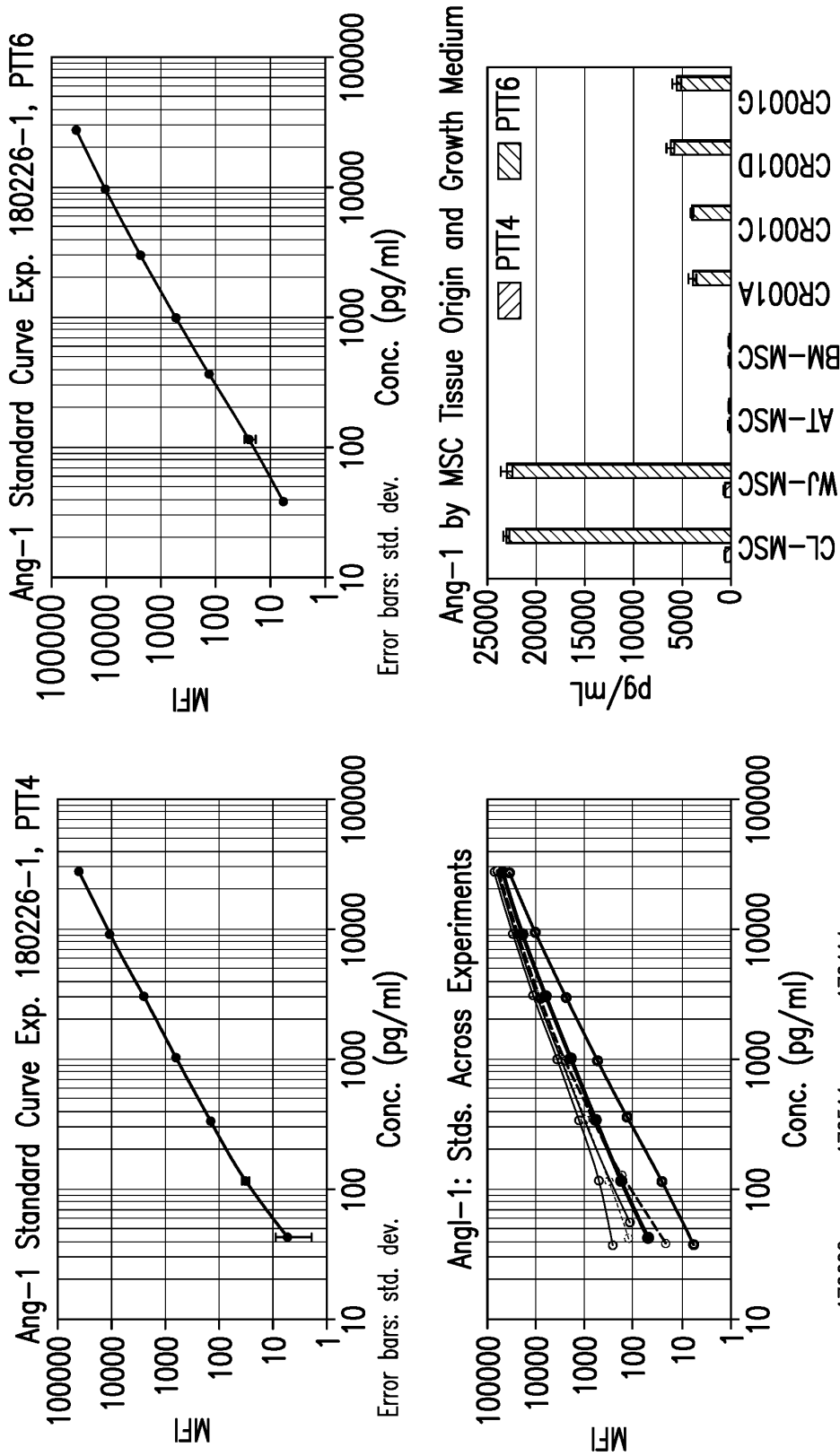


FIG.16

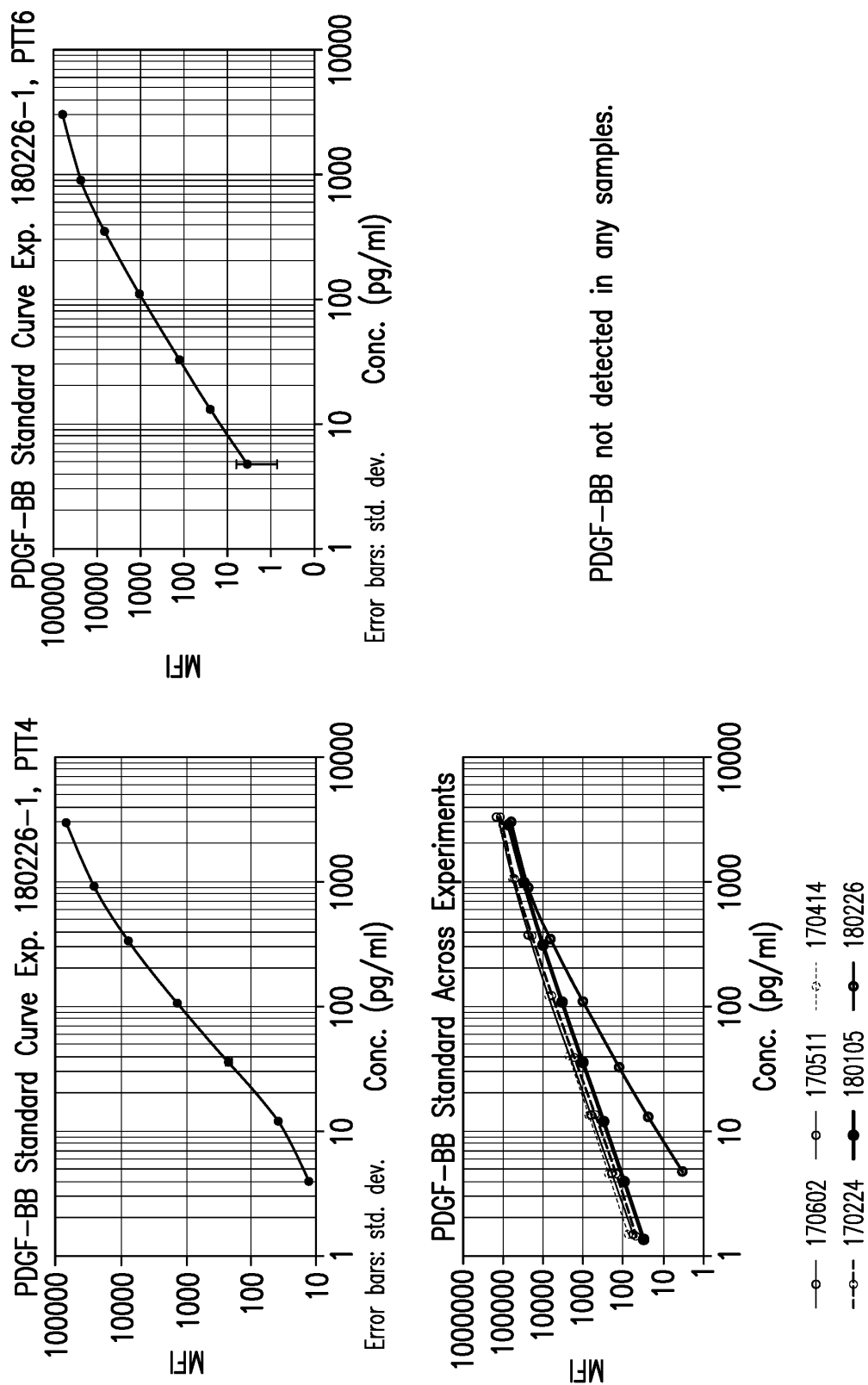


FIG.17

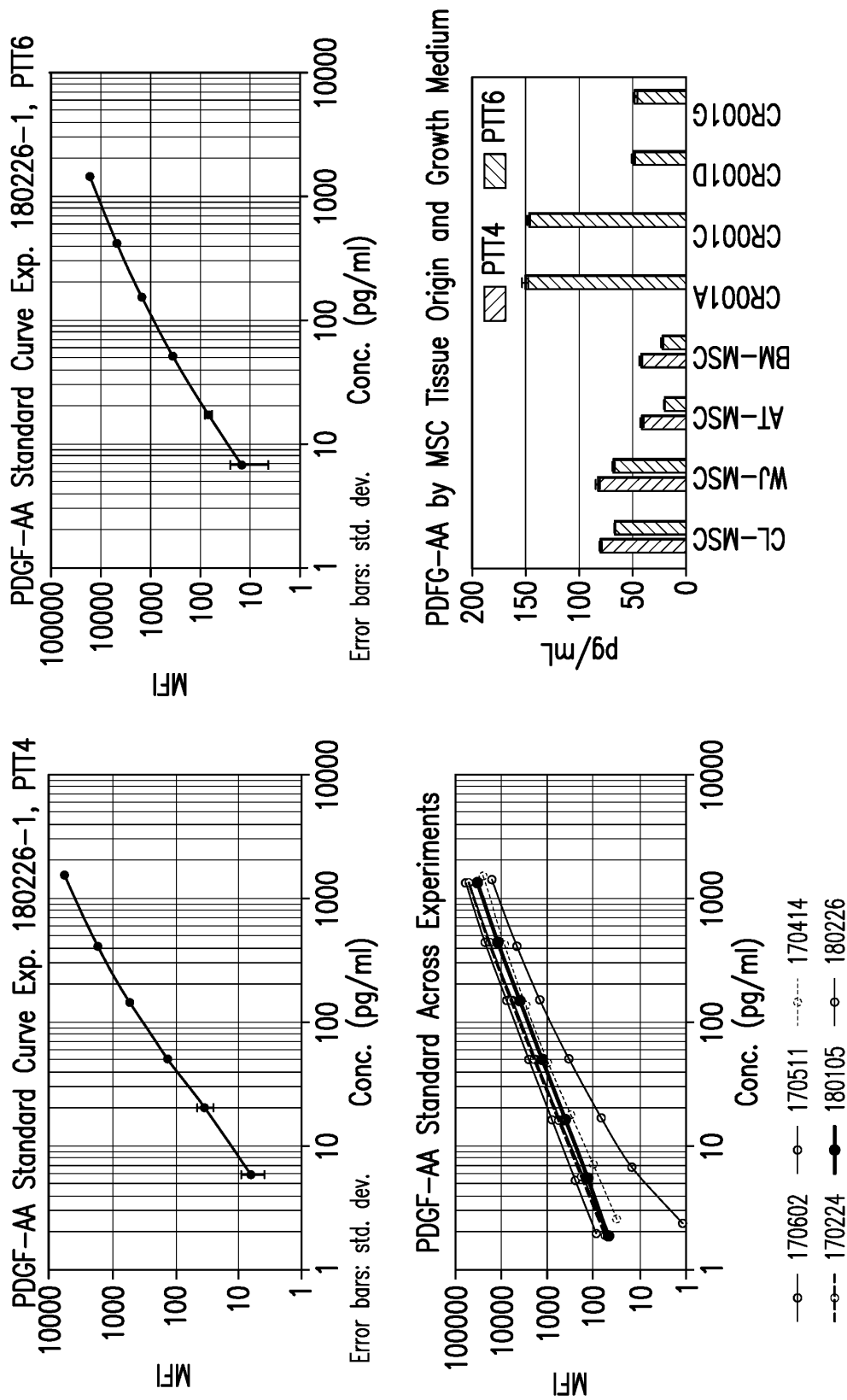
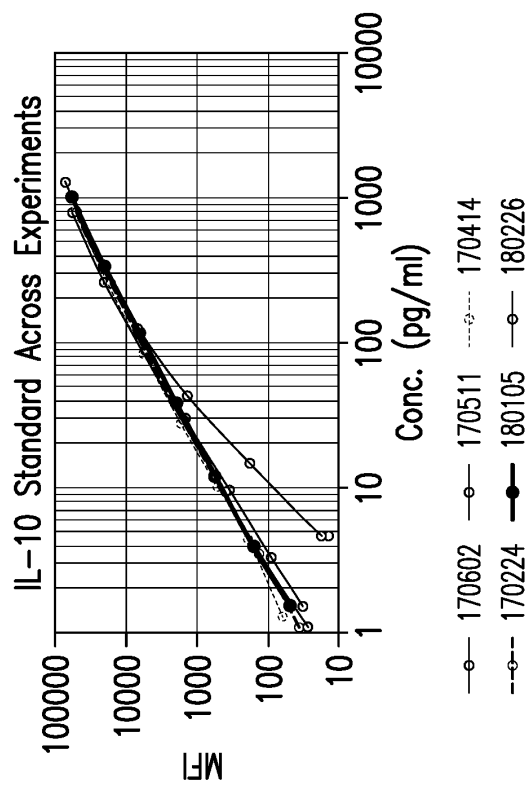
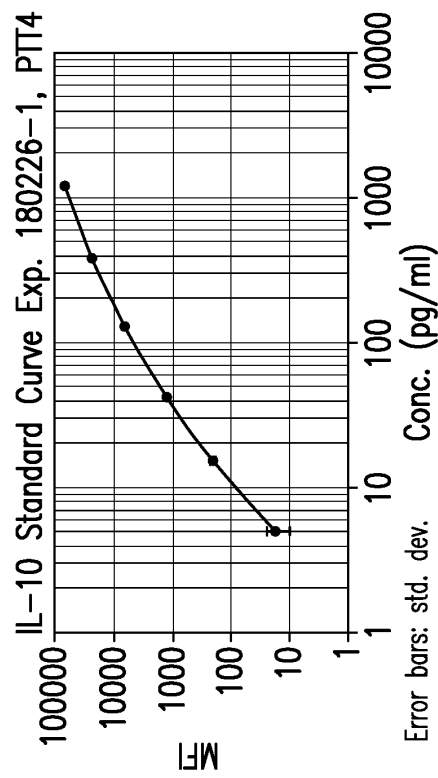
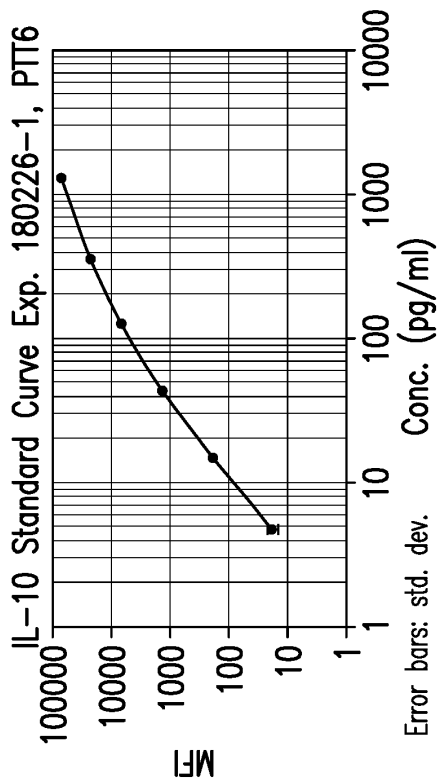


FIG.18



IL-10 not detected in any samples.

FIG.19

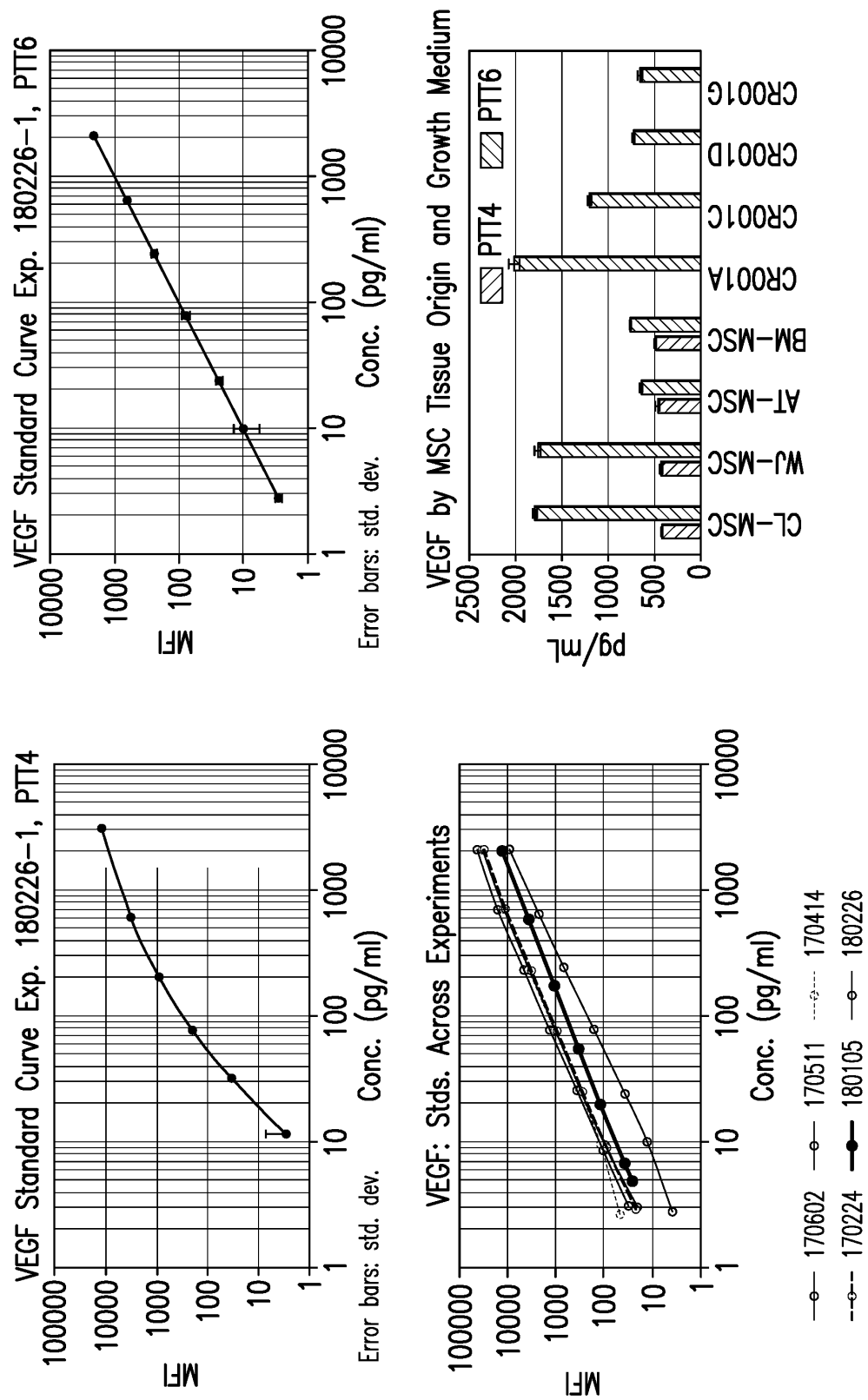


FIG.20

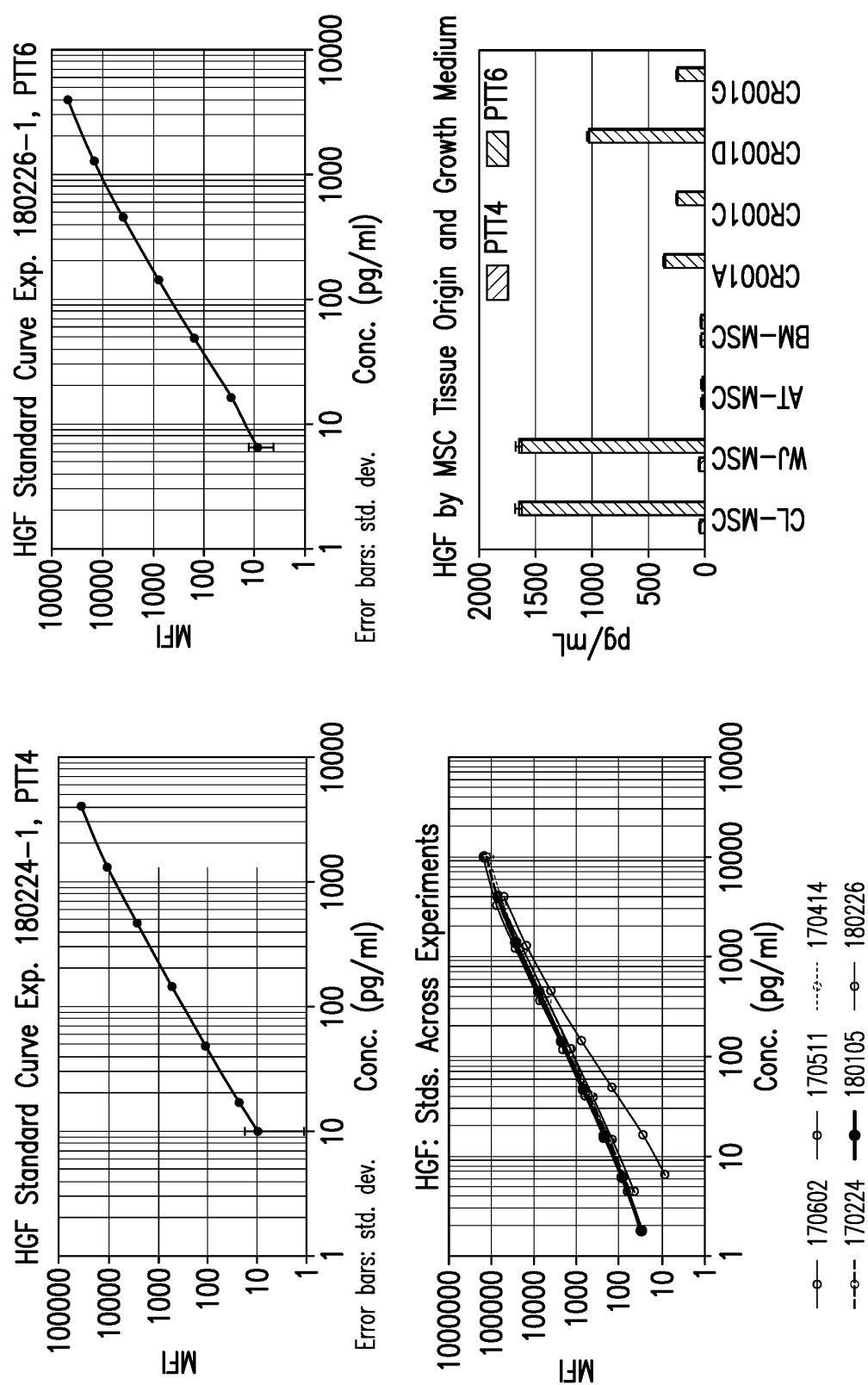


FIG.21

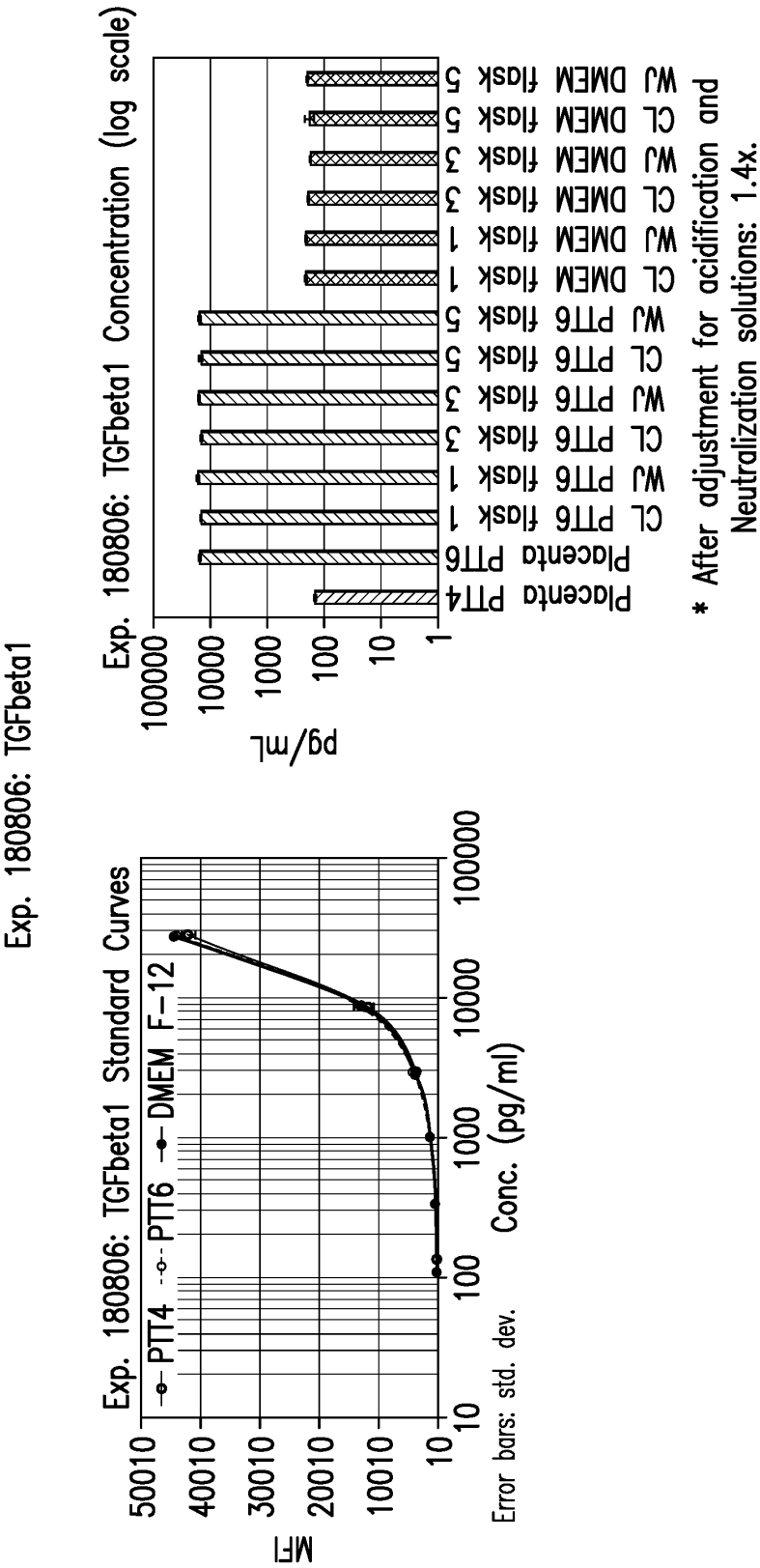
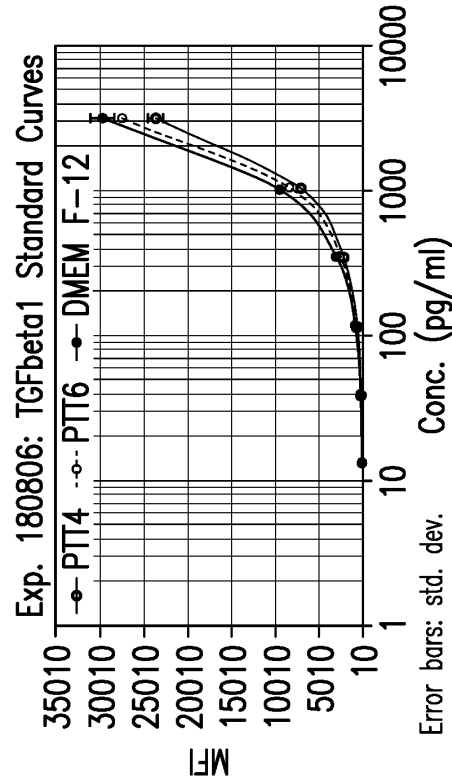


FIG.22

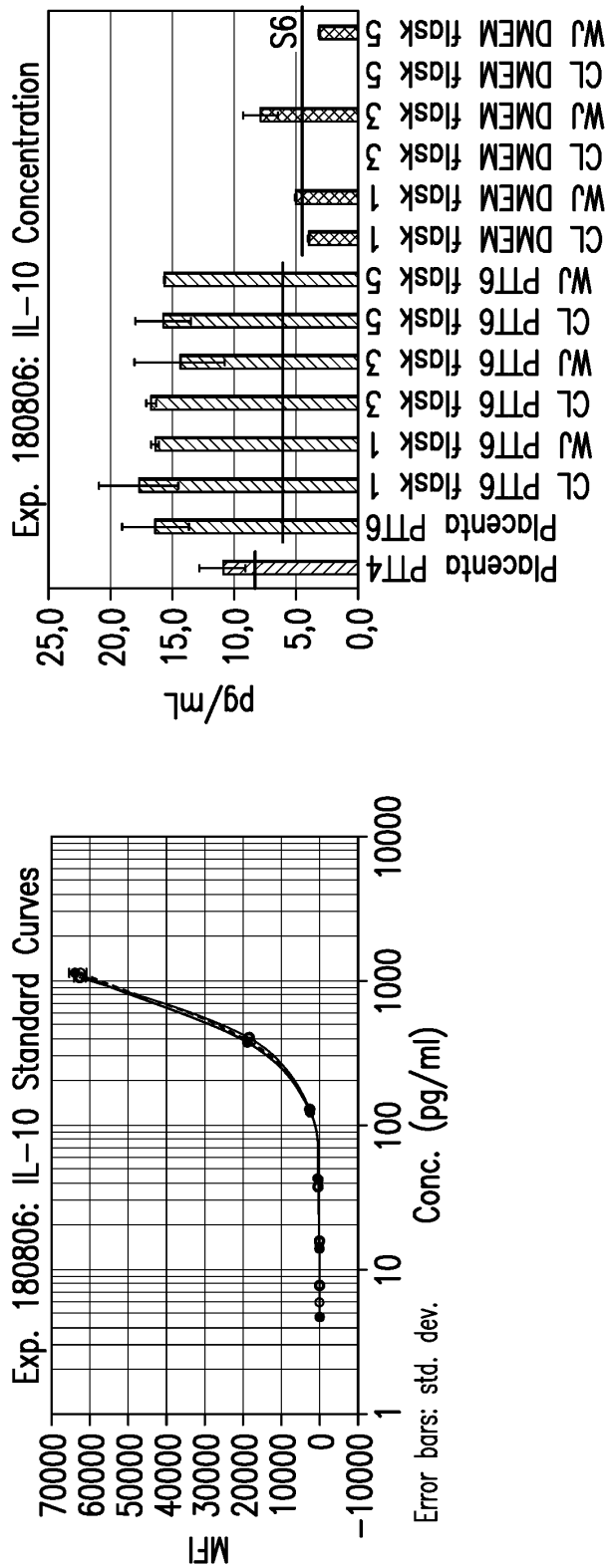
Exp. 180806: PDGF-BB



PDGF-BB below detection in all samples.

FIG.23

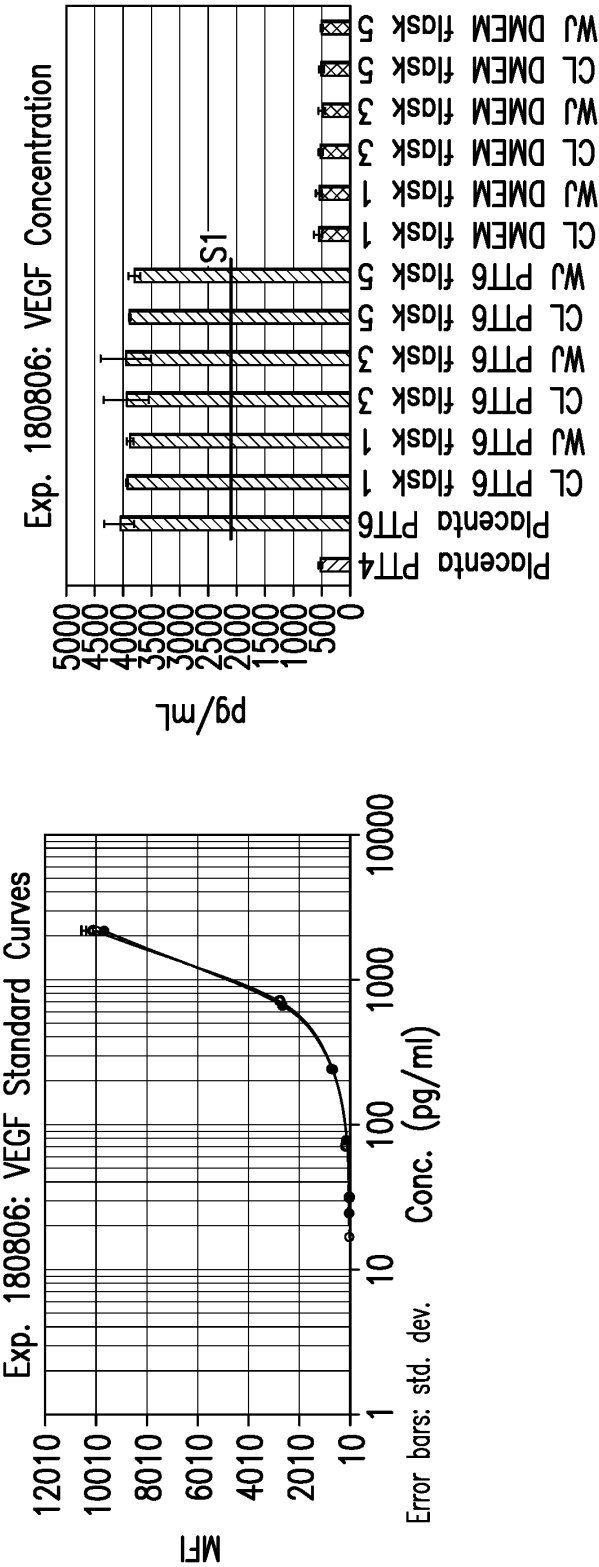
Exp. 180806: IL-10



S6 denotes the lowest standard used in the assay.
Any samples that fall below are considered below detection.

FIG.24

Exp. 180806: VEGF



S1 denotes the highest standard used in the assay. Any samples that fall above are considered extrapolated (too concentrated).

FIG.25

Exp. 180806: bFGF

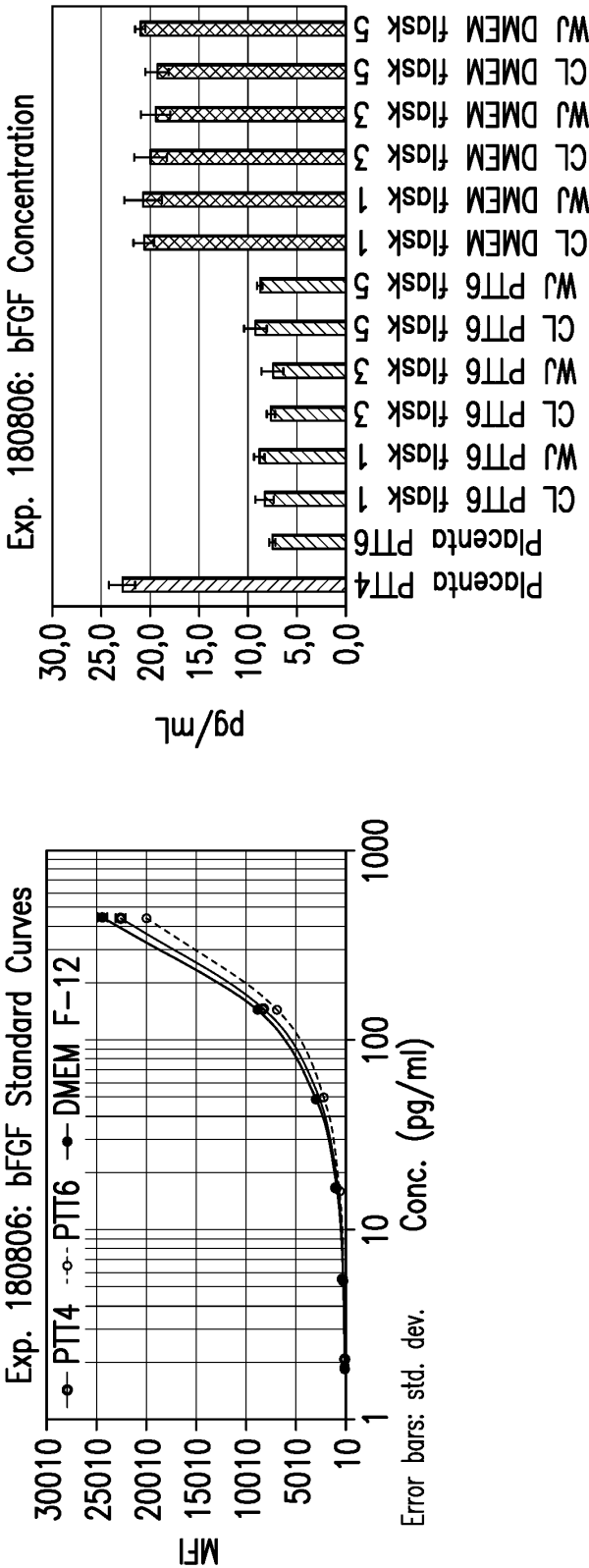
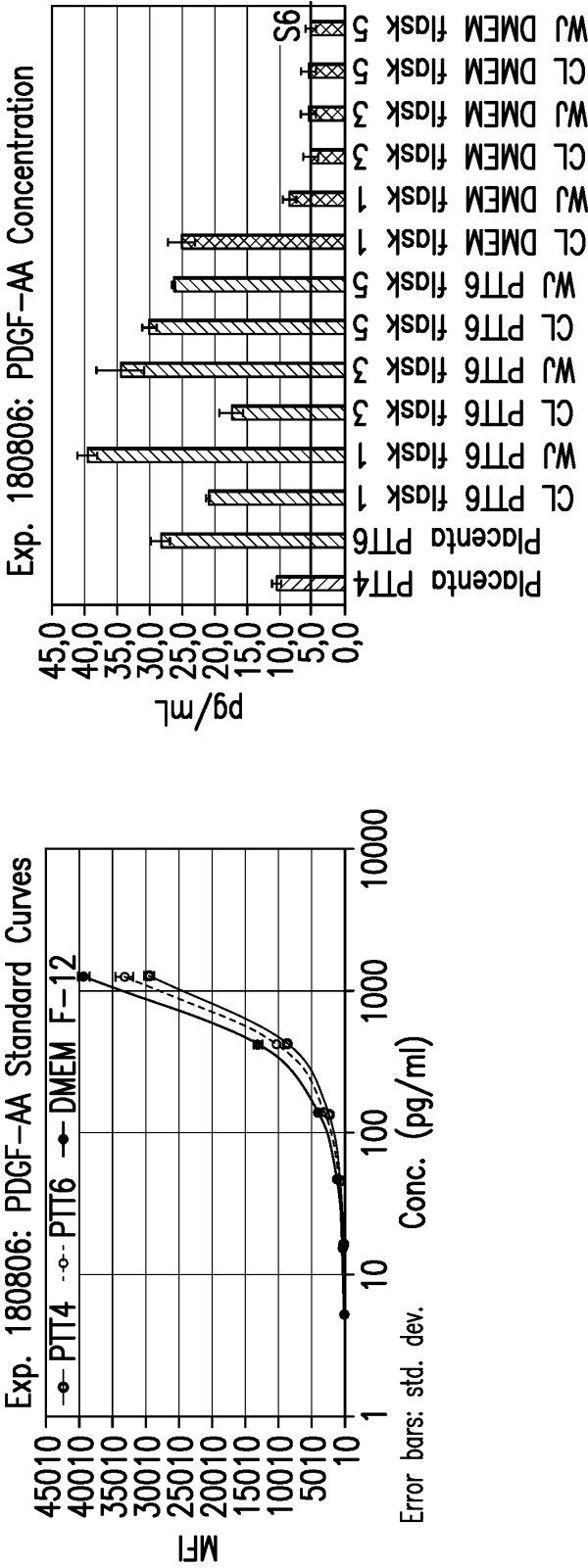


FIG.26

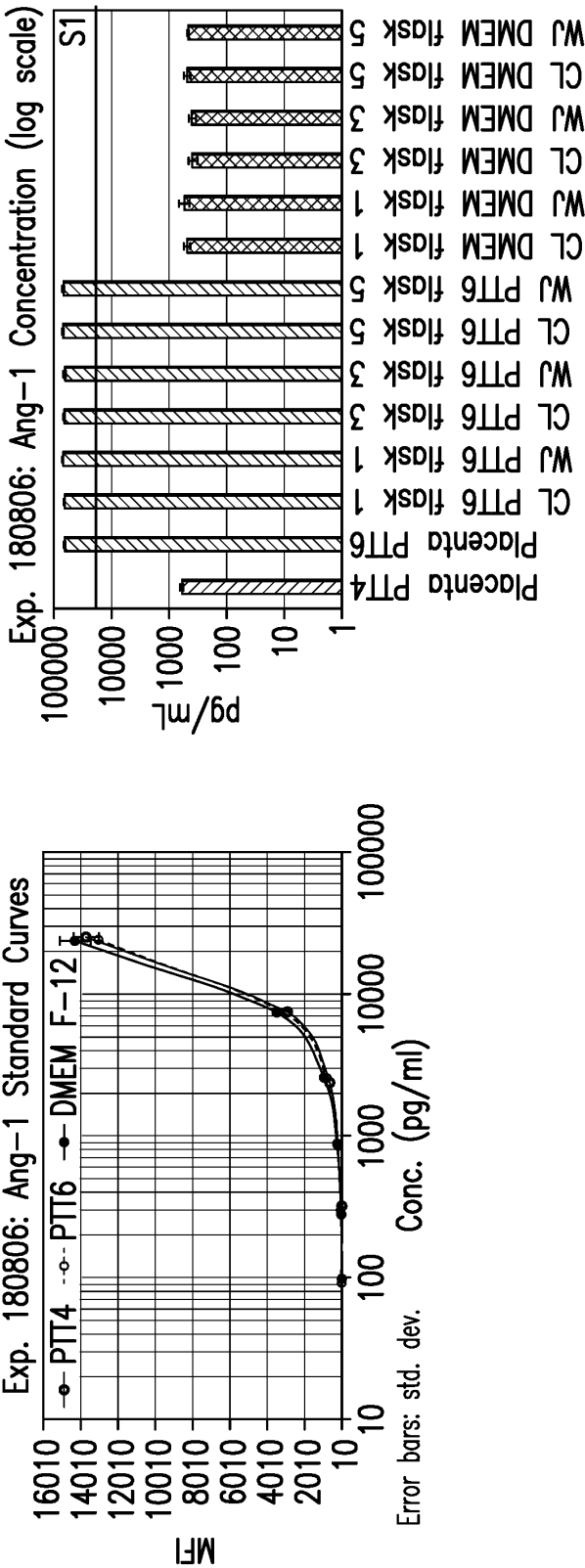
Exp. 180806: PDGF-AA



S6 denotes the lowest standard used in the assay.
Any samples that fall below are considered below detection.

FIG.27

Exp. 180806: Ang-1



S1 denotes the highest standard used in the assay.
Any samples that fall above are considered below extrapolated (too concentrated).

FIG.28

Exp. 180806: HGF

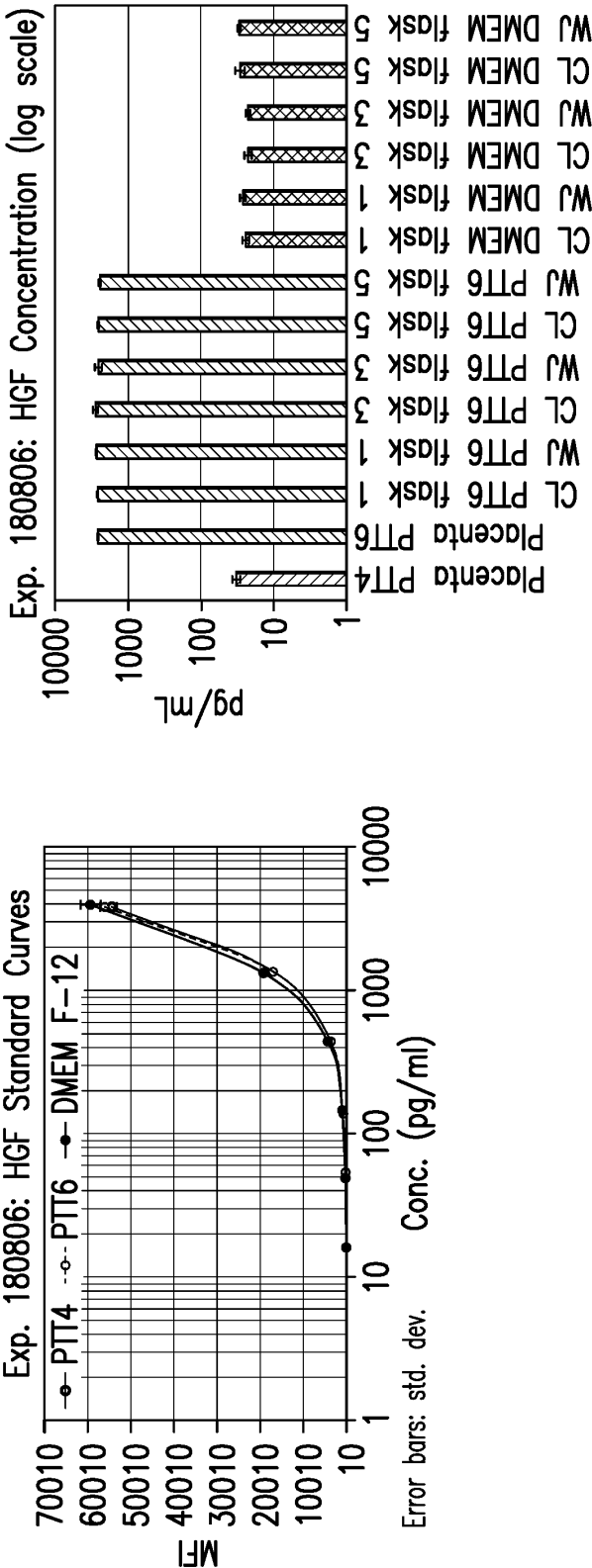


FIG.29