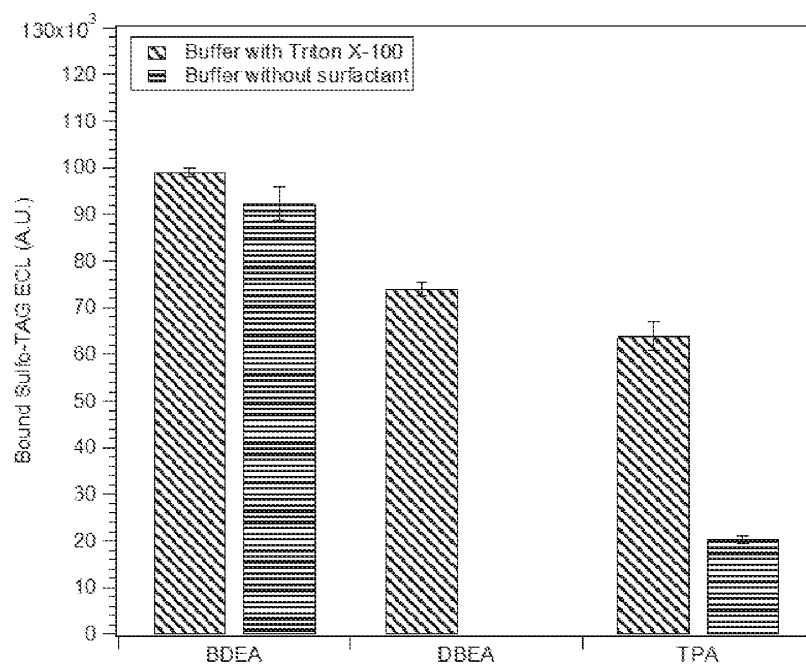
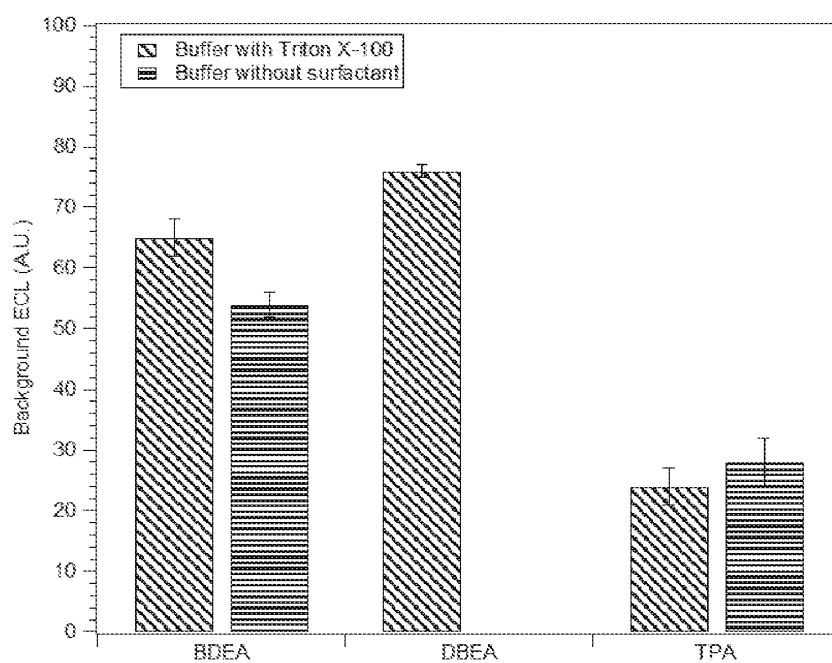


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**FIG. 1A****FIG. 1B**

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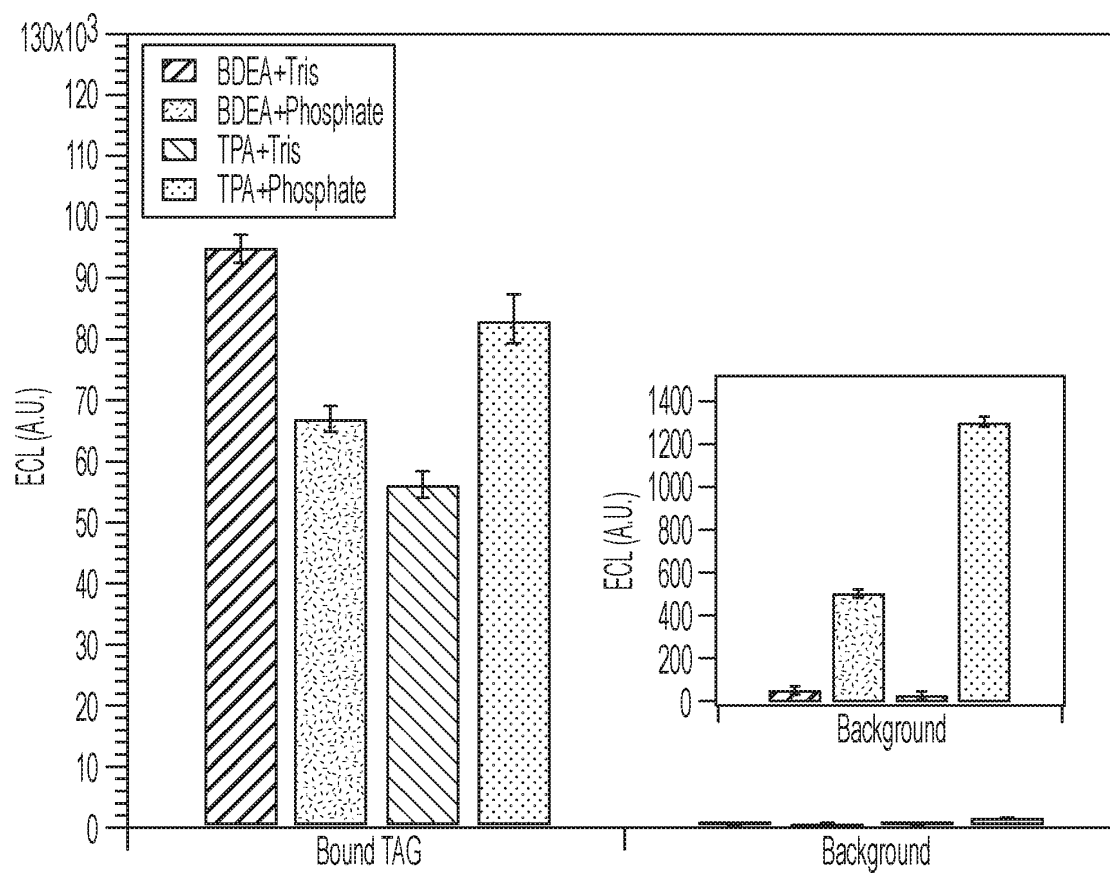


FIG. 1C

| Assay   | Signal        |        | Signal (Normalized) |      | CV (Intraplate) |       | CV (Interplate) |       |
|---------|---------------|--------|---------------------|------|-----------------|-------|-----------------|-------|
|         | TPA           | BDEA   | TPA                 | BDEA | TPA             | BDEA  | TPA             | BDEA  |
| Mid-Cal | IFN- $\gamma$ | 11,025 | 12,090              | 100% | 3.9%            | 3.3%  | 2.2%            | 3.0%  |
|         | IL-1 $\beta$  | 59,532 | 58,779              | 100% | 4.0%            | 3.8%  | 2.1%            | 1.9%  |
|         | IL-2          | 42,754 | 45,724              | 100% | 2.9%            | 2.7%  | 3.0%            | 2.7%  |
|         | IL-4          | 25,038 | 27,079              | 100% | 4.9%            | 4.4%  | 2.4%            | 1.4%  |
|         | IL-6          | 32,282 | 31,396              | 100% | 4.7%            | 4.2%  | 6.5%            | 4.6%  |
|         | IL-8          | 18,141 | 17,981              | 100% | 3.7%            | 3.8%  | 3.2%            | 4.9%  |
|         | IL-10         | 22,363 | 25,758              | 100% | 3.3%            | 2.9%  | 2.2%            | 1.7%  |
|         | IL12p70       | 10,515 | 10,829              | 100% | 6.6%            | 6.4%  | 3.7%            | 2.8%  |
|         | IL-13         | 7,977  | 11,148              | 100% | 5.5%            | 4.5%  | 3.2%            | 3.9%  |
|         | TNF- $\alpha$ | 8,639  | 8,329               | 100% | 4.4%            | 4.2%  | 2.6%            | 2.8%  |
| NSB     | Average       | 23,827 | 24,623              | 100% | 4.4%            | 4.0%  | 3.1%            | 3.0%  |
|         | IFN- $\gamma$ | 534    | 1,127               | 100% | 7.3%            | 6.4%  | 15.0%           | 20.9% |
|         | IL-1 $\beta$  | 1,788  | 5,321               | 100% | 7.3%            | 9.6%  | 13.8%           | 5.6%  |
|         | IL-2          | 321    | 592                 | 100% | 14.2%           | 6.6%  | 16.4%           | 10.9% |
|         | IL-4          | 175    | 262                 | 100% | 20.5%           | 19.9% | 20.5%           | 17.9% |
|         | IL-6          | 215    | 374                 | 100% | 12.4%           | 9.6%  | 13.4%           | 9.9%  |
|         | IL-8          | 272    | 421                 | 100% | 9.8%            | 9.7%  | 17.7%           | 17.0% |
|         | IL-10         | 456    | 884                 | 100% | 8.6%            | 6.4%  | 18.5%           | 19.0% |
|         | IL12p70       | 180    | 213                 | 100% | 35.8%           | 33.2% | 15.9%           | 22.8% |
|         | IL-13         | 155    | 245                 | 100% | 14.6%           | 12.9% | 12.4%           | 12.5% |
| NSB     | TNF- $\alpha$ | 189    | 331                 | 100% | 20.5%           | 11.7% | 14.9%           | 17.6% |
|         | Average       | 429    | 931                 | 100% | 15.1%           | 12.6% | 15.9%           | 15.4% |

FIG. 2A

| Assay   |               | Signal |           | Signal (Normalized) |           | CV (Intraplate) |           | CV (Interplate) |           |
|---------|---------------|--------|-----------|---------------------|-----------|-----------------|-----------|-----------------|-----------|
|         |               | TPA    | BDEA+NaCl | TPA                 | BDEA+NaCl | TPA             | BDEA+NaCl | TPA             | BDEA+NaCl |
| Mid-Cal | IFN- $\gamma$ | 8,494  | 7,774     | 100%                | 91.5%     | 3.3%            | 3.4%      | 4.2%            | 3.6%      |
|         | IL-1 $\beta$  | 49,770 | 39,585    | 100%                | 79.5%     | 3.3%            | 3.3%      | 3.5%            | 4.1%      |
|         | IL-2          | 40,845 | 34,365    | 100%                | 84.1%     | 2.8%            | 2.5%      | 3.2%            | 3.0%      |
|         | IL-4          | 31,722 | 28,372    | 100%                | 89.4%     | 4.4%            | 4.8%      | 4.7%            | 4.9%      |
|         | IL-6          | 33,504 | 26,074    | 100%                | 77.8%     | 4.1%            | 3.7%      | 6.3%            | 5.9%      |
|         | IL-8          | 16,029 | 12,887    | 100%                | 80.4%     | 3.5%            | 3.5%      | 3.6%            | 4.4%      |
|         | IL-10         | 22,983 | 21,797    | 100%                | 94.8%     | 3.4%            | 3.1%      | 3.9%            | 3.3%      |
|         | IL12p70       | 9,226  | 8,405     | 100%                | 91.1%     | 6.5%            | 6.1%      | 7.3%            | 7.3%      |
|         | IL-13         | 10,451 | 11,703    | 100%                | 112.0%    | 4.9%            | 3.8%      | 5.1%            | 4.0%      |
|         | TNF- $\alpha$ | 9,048  | 7,132     | 100%                | 78.8%     | 3.9%            | 3.5%      | 4.3%            | 3.5%      |
| NSB     | Average       | 23,207 | 19,809    | 100%                | 88.0%     | 4.0%            | 3.8%      | 4.6%            | 4.4%      |
|         | IFN- $\gamma$ | 242    | 367       | 100%                | 151.7%    | 13.6%           | 9.9%      | 13.9%           | 12.4%     |
|         | IL-1 $\beta$  | 1,374  | 1,757     | 100%                | 127.9%    | 9.1%            | 8.7%      | 9.7%            | 18.3%     |
|         | IL-2          | 231    | 214       | 100%                | 92.5%     | 12.8%           | 14.7%     | 12.8%           | 15.9%     |
|         | IL-4          | 124    | 117       | 100%                | 94.4%     | 27.6%           | 28.2%     | 28.1%           | 30.1%     |
|         | IL-6          | 148    | 160       | 100%                | 108.4%    | 16.2%           | 14.6%     | 16.7%           | 15.8%     |
|         | IL-8          | 165    | 172       | 100%                | 104.0%    | 13.9%           | 13.5%     | 14.8%           | 13.7%     |
|         | IL-10         | 198    | 245       | 100%                | 123.6%    | 14.8%           | 13.2%     | 15.0%           | 14.3%     |
|         | IL12p70       | 151    | 172       | 100%                | 113.9%    | 17.4%           | 17.2%     | 17.7%           | 17.8%     |
|         | IL-13         | 120    | 115       | 100%                | 85.8%     | 19.9%           | 21.1%     | 20.3%           | 21.8%     |
| NSB     | TNF- $\alpha$ | 202    | 244       | 100%                | 120.7%    | 14.4%           | 13.9%     | 15.1%           | 14.4%     |
|         | Average       | 296    | 356       | 100%                | 113.3%    | 16.0%           | 15.5%     | 16.4%           | 17.4%     |

FIG. 2B

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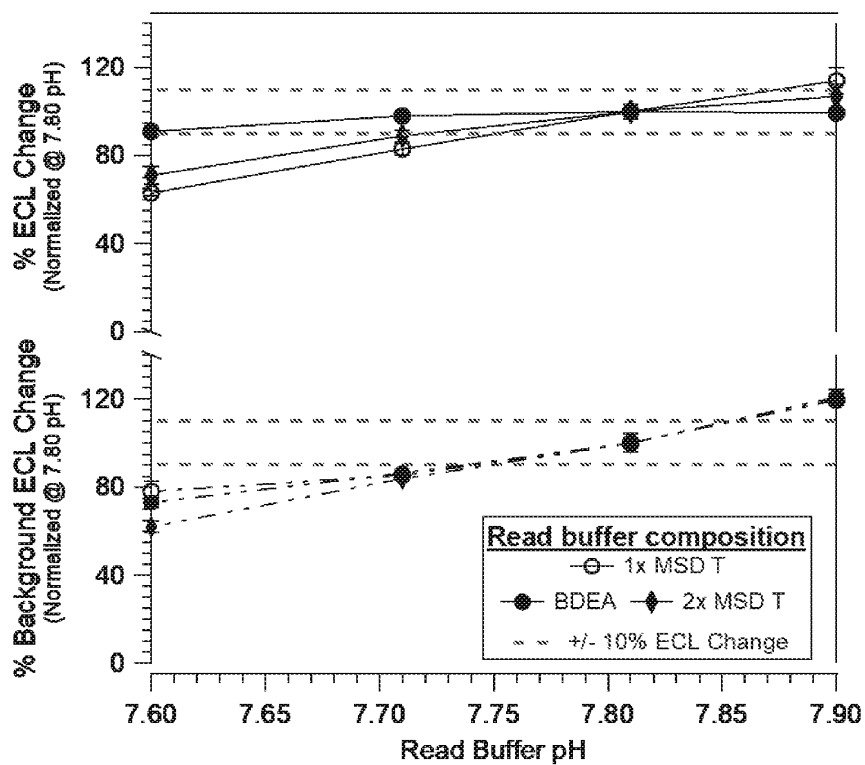


FIG. 3A

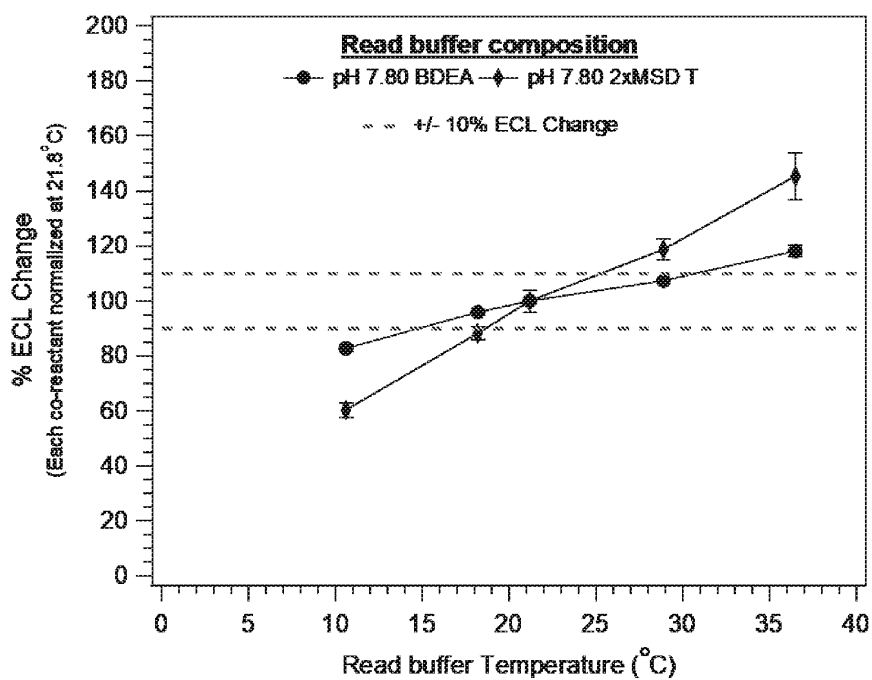


FIG. 3B

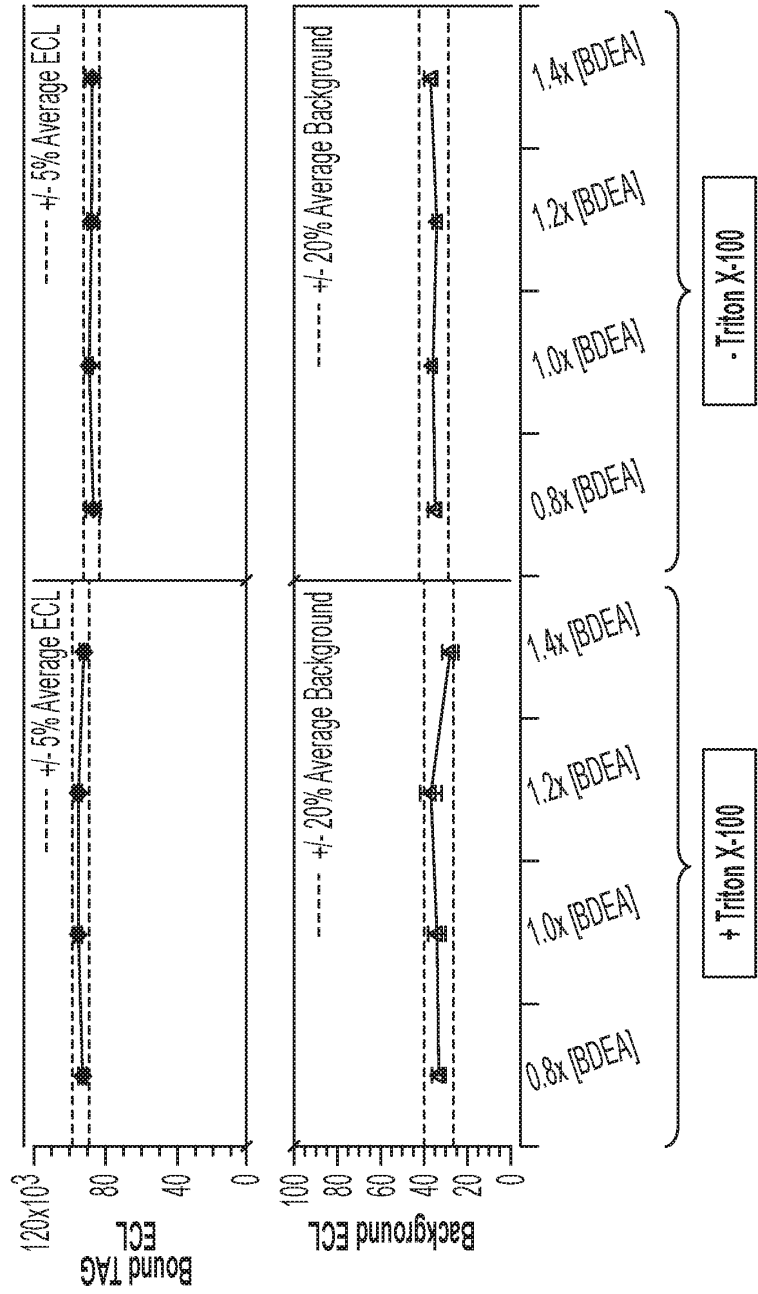
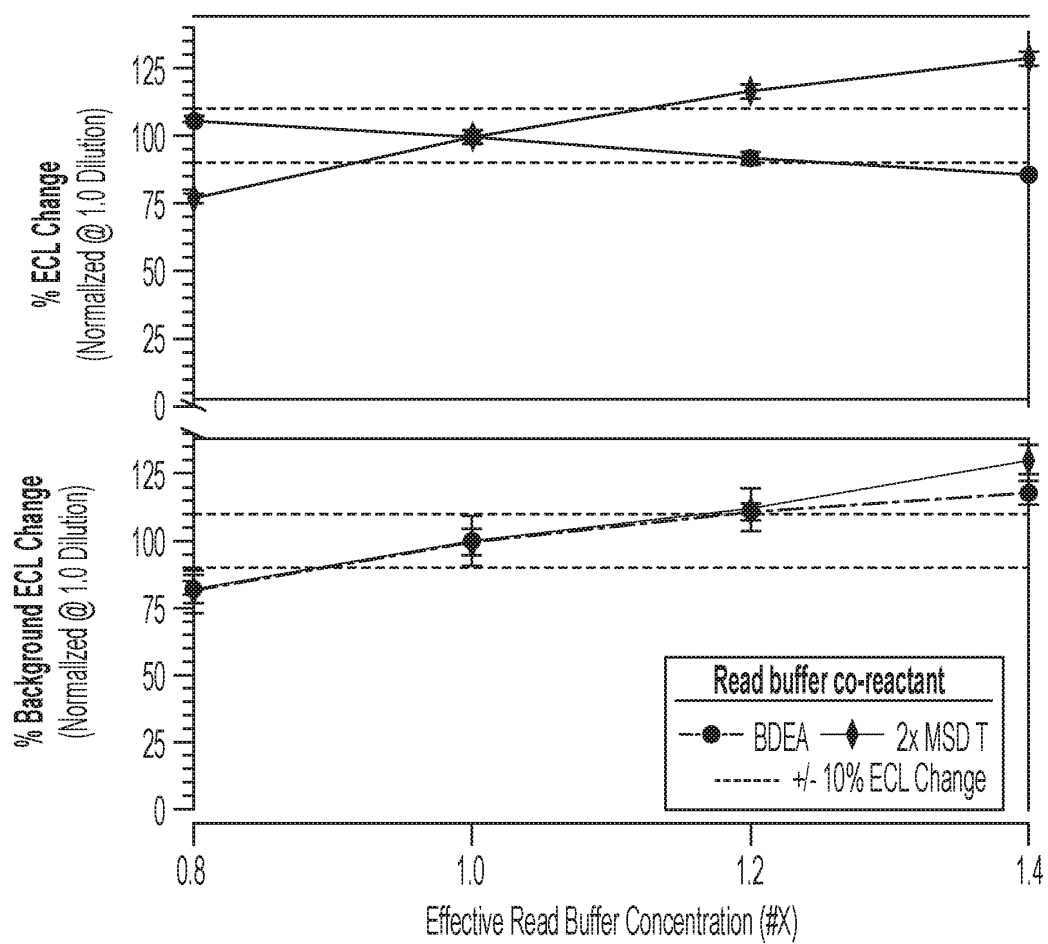


FIG. 3C

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**FIG. 3D**

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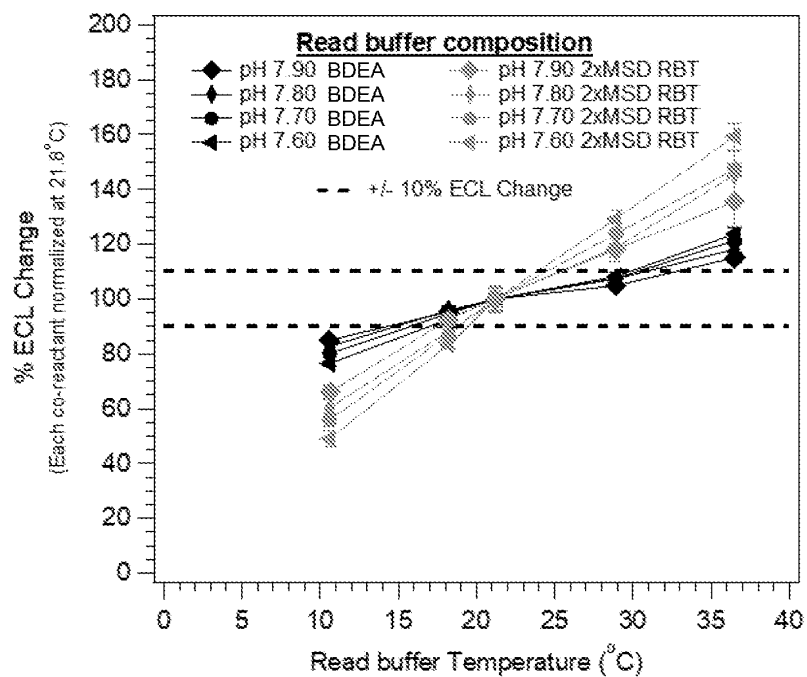


FIG. 3E

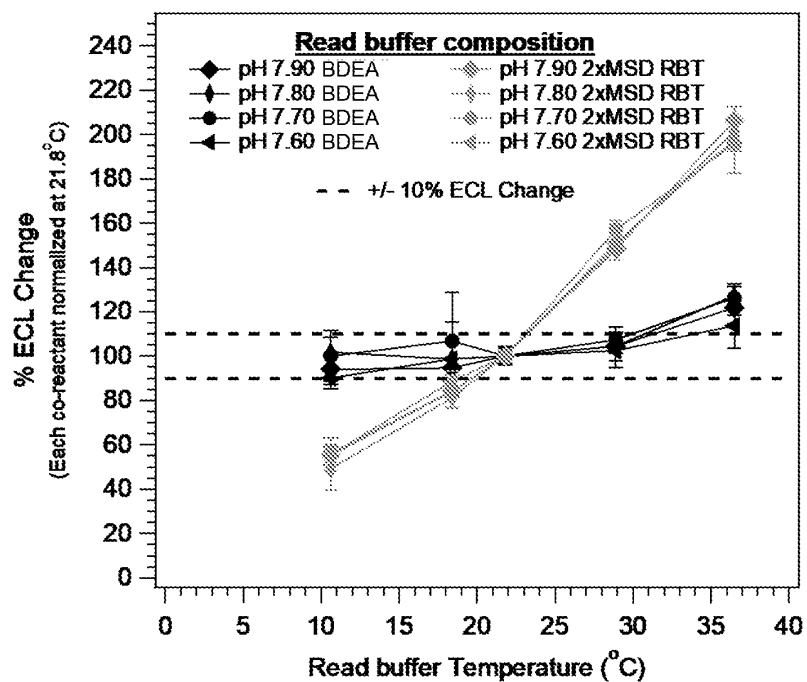


FIG. 3F

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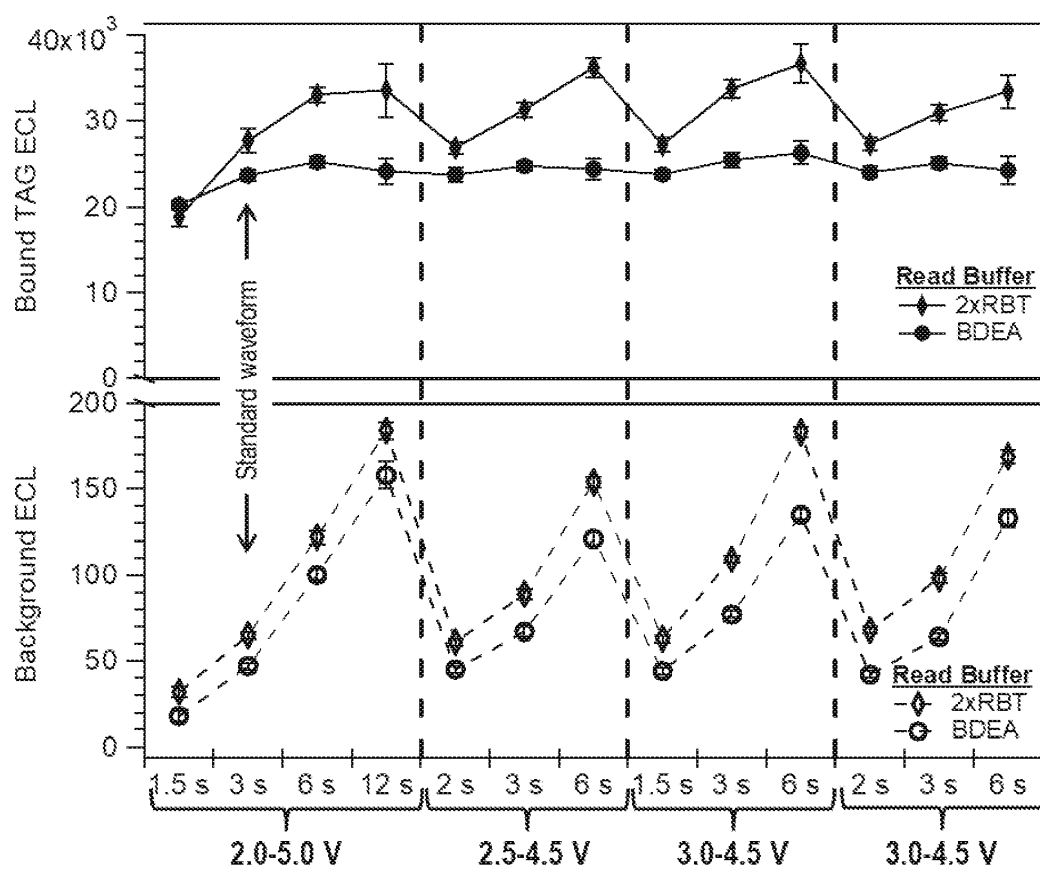
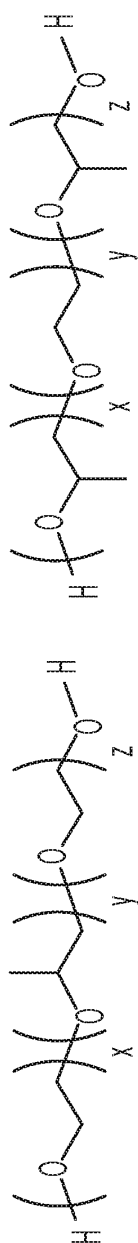


FIG. 4

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## Surfactant chemical structures



Pluronic 31R1

General structures

- Koliphor P 407
- Pluronic P-123
- Pluronic L-121

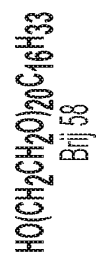
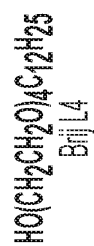
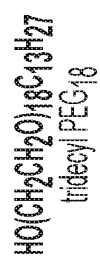


FIG. 5A

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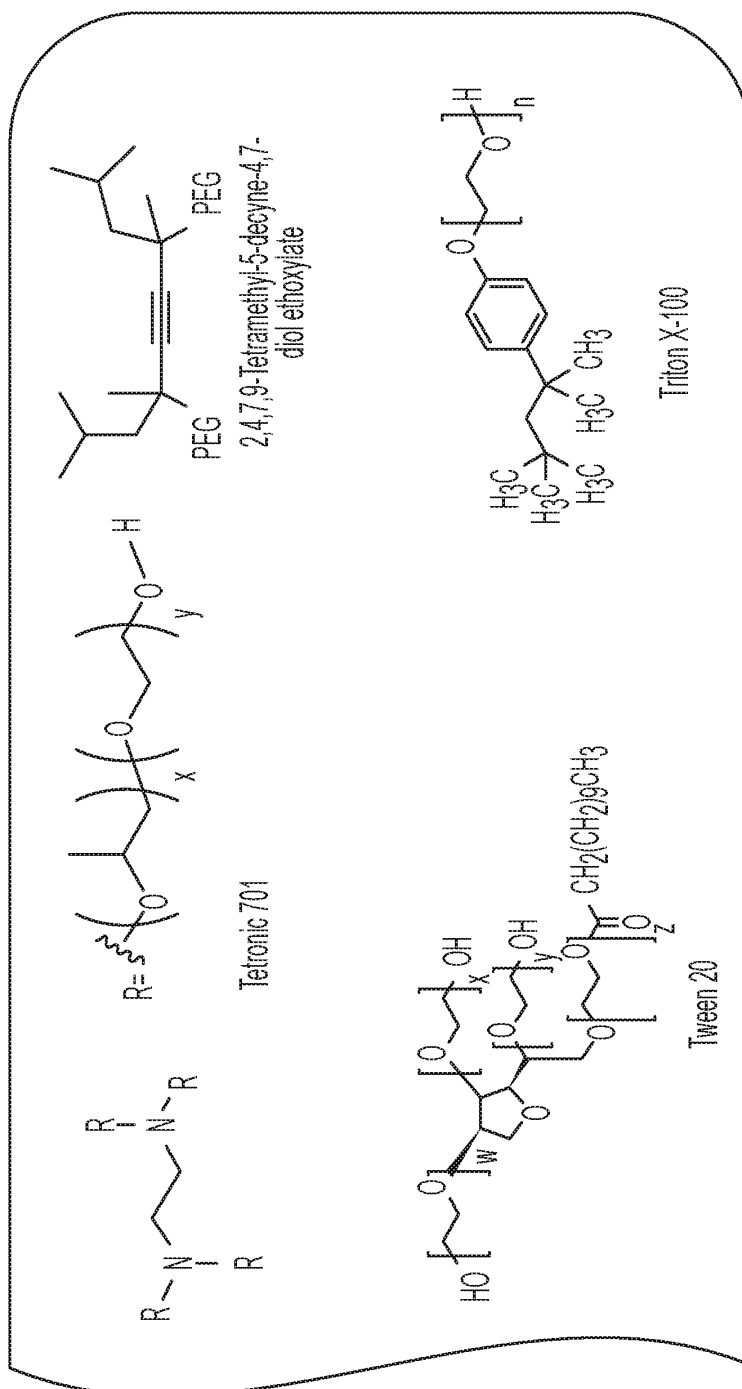


FIG. 5A (Continued)

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### Read Buffer liquid/air interfacial tensions

| Surfactant in BDEA Read Buffer | Average $\gamma_{l/a}$ (dyn/cm) | STDEV |
|--------------------------------|---------------------------------|-------|
| Surfactant free                | 53.19                           | 0.59  |
| L-121                          | 42.06                           | 0.23  |
| P407                           | 37.19                           | 0.24  |
| Tween 20                       | 34.16                           | 0.33  |
| Brij 58                        | 34.05                           | 0.43  |
| Decyne-3,4-diol                | 33.46                           | 0.09  |
| 31R1                           | 32.36                           | 0.05  |
| Brij L4 (t=35 s)               | 32.30                           | 0.21  |
| Tetronic 701                   | 31.20                           | 0.11  |
| P-123                          | 29.60                           | 0.12  |
| PEG(18)-tridecyl               | 29.39                           | 0.08  |
| Triton X-100                   | 27.20                           | 0.10  |

**FIG. 5B**

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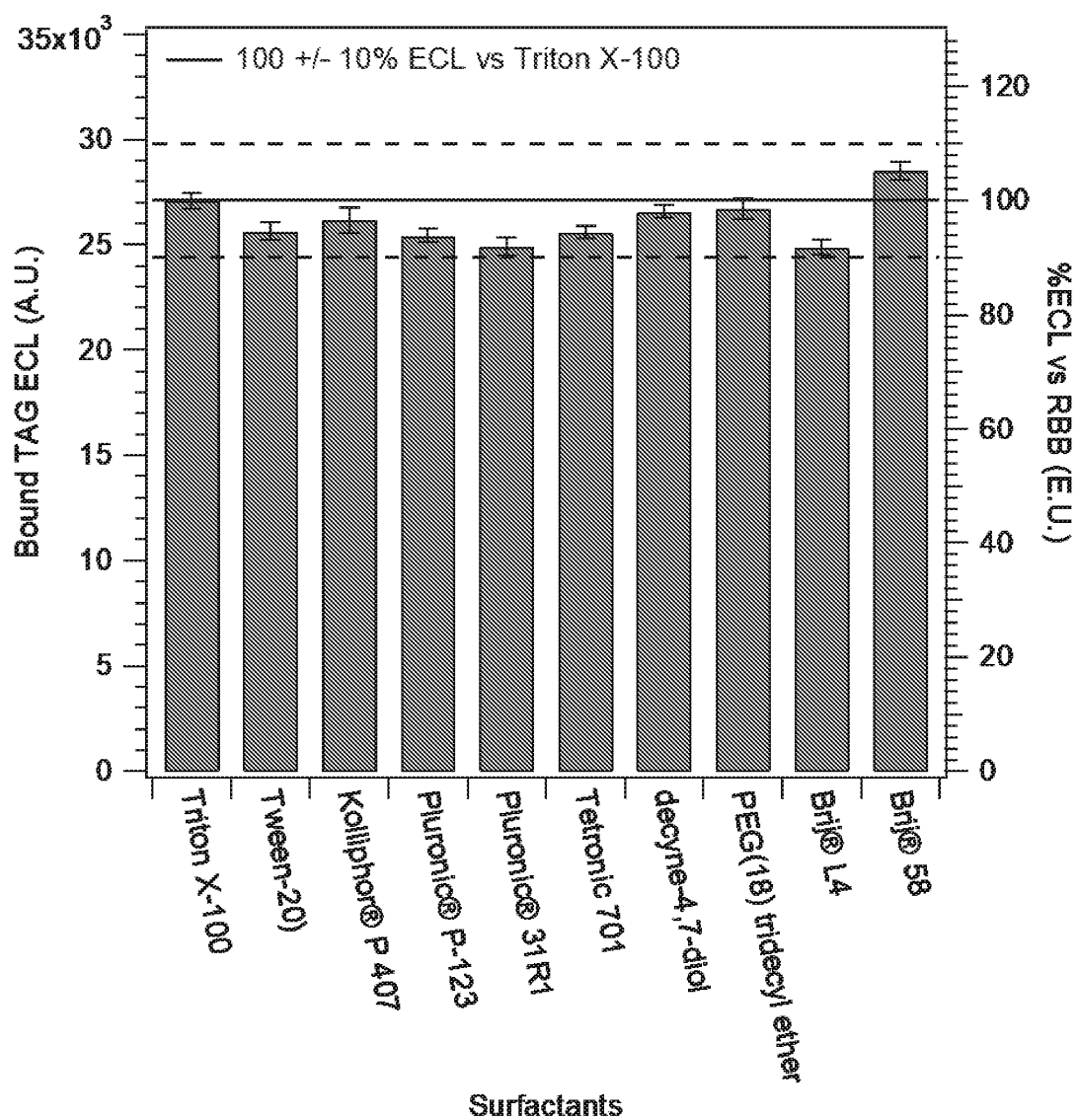


FIG. 6A

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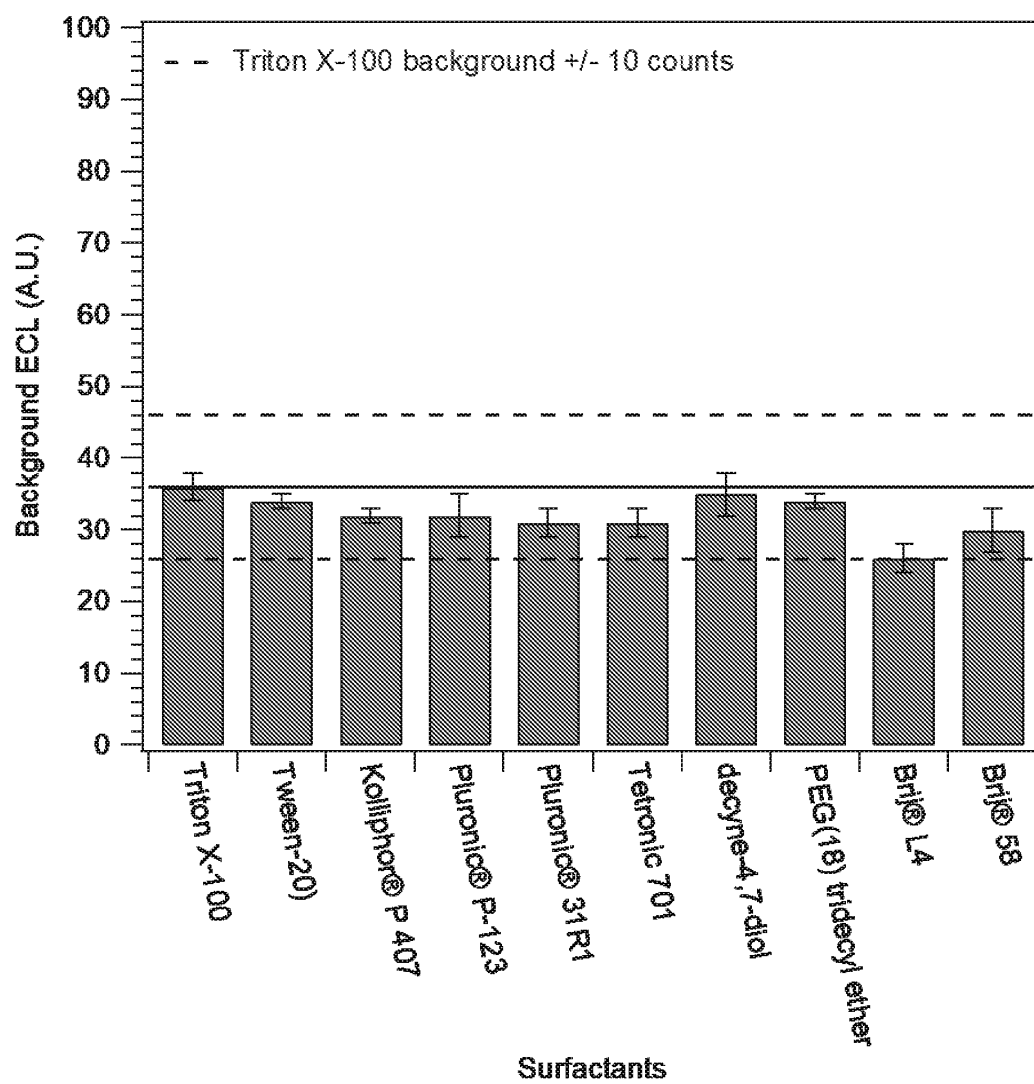


FIG. 6B

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| EV Assay | Surfactant in BDEA Read Buffer                   | Soak Time | ECL   | %CV (n=4) | %ECL vs Tween- 20<br>@ t=0 min | %ECL vs soak<br>@ t=15 min |
|----------|--------------------------------------------------|-----------|-------|-----------|--------------------------------|----------------------------|
| CD9/CD9  | Tween 20                                         | 0 min     | 61933 | 4.1%      | 100.0%                         | 103.9%                     |
|          |                                                  | 15 min    | 64326 | 3.9%      |                                |                            |
|          | Triton X-100                                     | 0 min     | 809   | 8.7%      | 1.3%                           | 50.4%                      |
|          |                                                  | 15 min    | 408   | 6.8%      |                                |                            |
|          | Kolliphor® P 407                                 | 0 min     | 58851 | 5.1%      | 95.0%                          | 109.2%                     |
|          |                                                  | 15 min    | 64275 | 1.4%      |                                |                            |
|          | Pluronic® P-123                                  | 0 min     | 61155 | 3.1%      | 98.7%                          | 99.9%                      |
|          |                                                  | 15 min    | 61080 | 3.4%      |                                |                            |
|          | Pluronic® L-121                                  | 0 min     | 64643 | 3.1%      | 104.4%                         | 104.1%                     |
|          |                                                  | 15 min    | 67263 | 2.3%      |                                |                            |
|          | Pluronic® 31R1                                   | 0 min     | 63193 | 2.9%      | 102.0%                         | 96.5%                      |
|          |                                                  | 15 min    | 60963 | 3.6%      |                                |                            |
|          | Tetronic 701                                     | 0 min     | 59875 | 0.9%      | 96.7%                          | 101.9%                     |
|          |                                                  | 15 min    | 61002 | 5.2%      |                                |                            |
|          | 2,4,7,9-Tetramethyl-5-decyne-4,7-diol ethoxylate | 0 min     | 60697 | 1.3%      | 98.0%                          | 104.6%                     |
|          |                                                  | 15 min    | 63506 | 4.4%      |                                |                            |
|          | PEG(18) tridecyl ether                           | 0 min     | 62710 | 2.2%      | 101.3%                         | 105.1%                     |
|          |                                                  | 15 min    | 65897 | 4.3%      |                                |                            |
|          | Brij® L4                                         | 0 min     | 60163 | 2.0%      | 97.1%                          | 99.6%                      |
|          |                                                  | 15 min    | 59923 | 1.9%      |                                |                            |
|          | Brij® C10                                        | 0 min     | 49150 | 5.7%      | 79.4%                          | 43.7%                      |
|          |                                                  | 15 min    | 21455 | 9.8%      |                                |                            |
|          | Brij® 58                                         | 0 min     | 60936 | 4.2%      | 98.4%                          | 95.1%                      |
|          |                                                  | 15 min    | 57960 | 3.2%      |                                |                            |
|          | Brij® S20                                        | 0 min     | 62097 | 2.1%      | 100.3%                         | 87.0%                      |
|          |                                                  | 15 min    | 54053 | 3.9%      |                                |                            |
|          | Brij® S10                                        | 0 min     | 63885 | 4.3%      | 103.2%                         | 78.9%                      |
|          |                                                  | 15 min    | 50430 | 2.5%      |                                |                            |

FIG. 7

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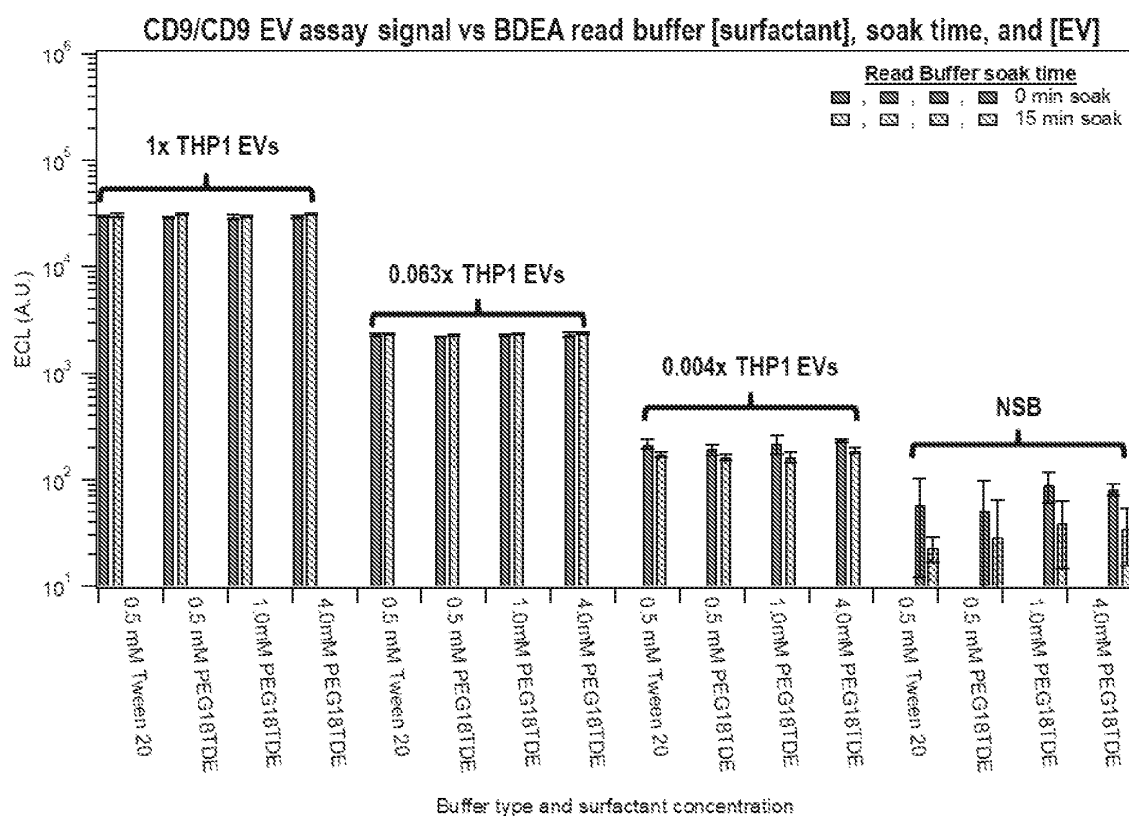


FIG. 8

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|            |            | Average %ECL loss over 39-652 pg/ml cal |       |        |       |       |        |
|------------|------------|-----------------------------------------|-------|--------|-------|-------|--------|
|            |            | MSD T2x                                 |       |        | BDEA  |       |        |
| Capture    | Detect     | 0 min                                   | 5 min | 10 min | 0 min | 5 min | 10 min |
| 4880-B18-1 | 4883-C28-2 | 0%                                      | 41%   | 63%    | 0%    | 26%   | 43%    |
| 4883-C28-2 | 4880-B18-1 | 0%                                      | 20%   | 32%    | 0%    | 2%    | 2%     |
| 4883-C28-2 | 4882-A54-1 | 0%                                      | 46%   | 68%    | 0%    | 46%   | 69%    |
| 4883-C69-2 | 4882-A54-1 | 0%                                      | 48%   | 69%    | 0%    | 51%   | 67%    |
| 4883-C69-2 | 4883-G74-3 | 0%                                      | 17%   | 33%    | 0%    | 8%    | 15%    |
| 4883-C69-2 | 4883-J01-1 | 0%                                      | 38%   | 60%    | 0%    | 32%   | 53%    |

FIG. 9A

|             |            | Average %ECL loss over 10-156 pg/ml cal |       |        |       |       |        |
|-------------|------------|-----------------------------------------|-------|--------|-------|-------|--------|
|             |            | MSD T2x                                 |       |        | BDEA  |       |        |
| Capture     | Detect     | 0 min                                   | 5 min | 10 min | 0 min | 5 min | 10 min |
| 512004      | 507602     | 0%                                      | 9%    | 19%    | 0%    | 3%    | 6%     |
| 512004      | 4390-B35-1 | 0%                                      | 66%   | 80%    | 0%    | 54%   | 71%    |
| 512004      | 558088     | 0%                                      | 11%   | 20%    | 0%    | 3%    | 10%    |
| 507602      | 512004     | 0%                                      | 6%    | 12%    | 0%    | 1%    | 4%     |
| CABT-WN1726 | 4390-B35-1 | 0%                                      | 71%   | 83%    | 0%    | 59%   | 73%    |
| CABT-WN1726 | 507602     | 0%                                      | 8%    | 19%    | 0%    | -1%   | 3%     |
| CABT-WN1726 | 4390-F63-4 | 0%                                      | 51%   | 72%    | 0%    | 34%   | 54%    |
| 130-096-860 | 4390-B35-1 | 0%                                      | 68%   | 80%    | 0%    | 54%   | 69%    |
| 4365-A26-3  | 4365-A95-1 | 0%                                      | 33%   | 54%    | 0%    | 21%   | 39%    |

FIG. 9B

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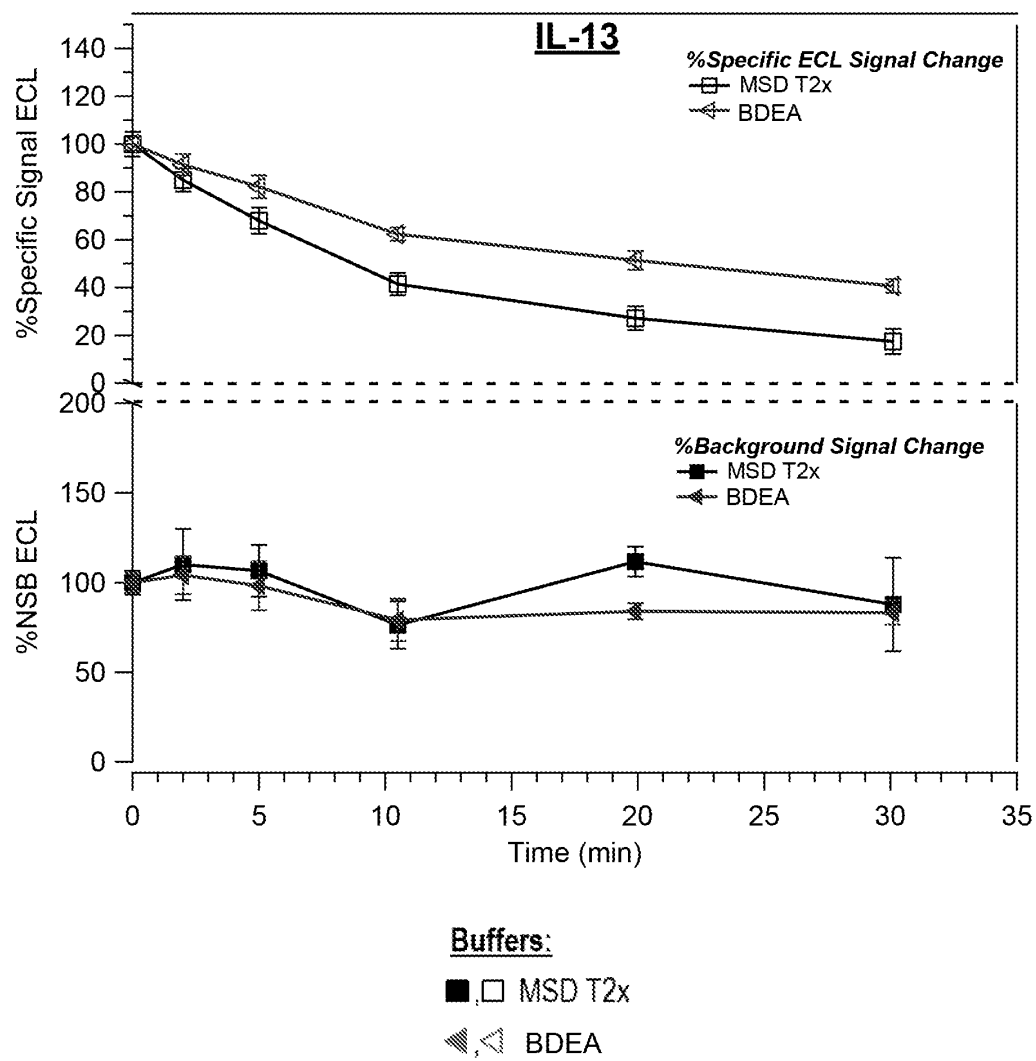
|            |            | Average %ECL loss over 195 pg/ml cal |       |        |       |       |        |
|------------|------------|--------------------------------------|-------|--------|-------|-------|--------|
|            |            | MSD T2x                              |       |        | BDEA  |       |        |
| Capture    | Detect     | 0 min                                | 5 min | 10 min | 0 min | 5 min | 10 min |
| 4945-A82-7 | AF1750     | 0%                                   | 43%   | 61%    | 0%    | 11%   | 28%    |
| 4945-A82-7 | 4942-D39-6 | 0%                                   | 43%   | 63%    | 0%    | 11%   | 24%    |

FIG. 9C

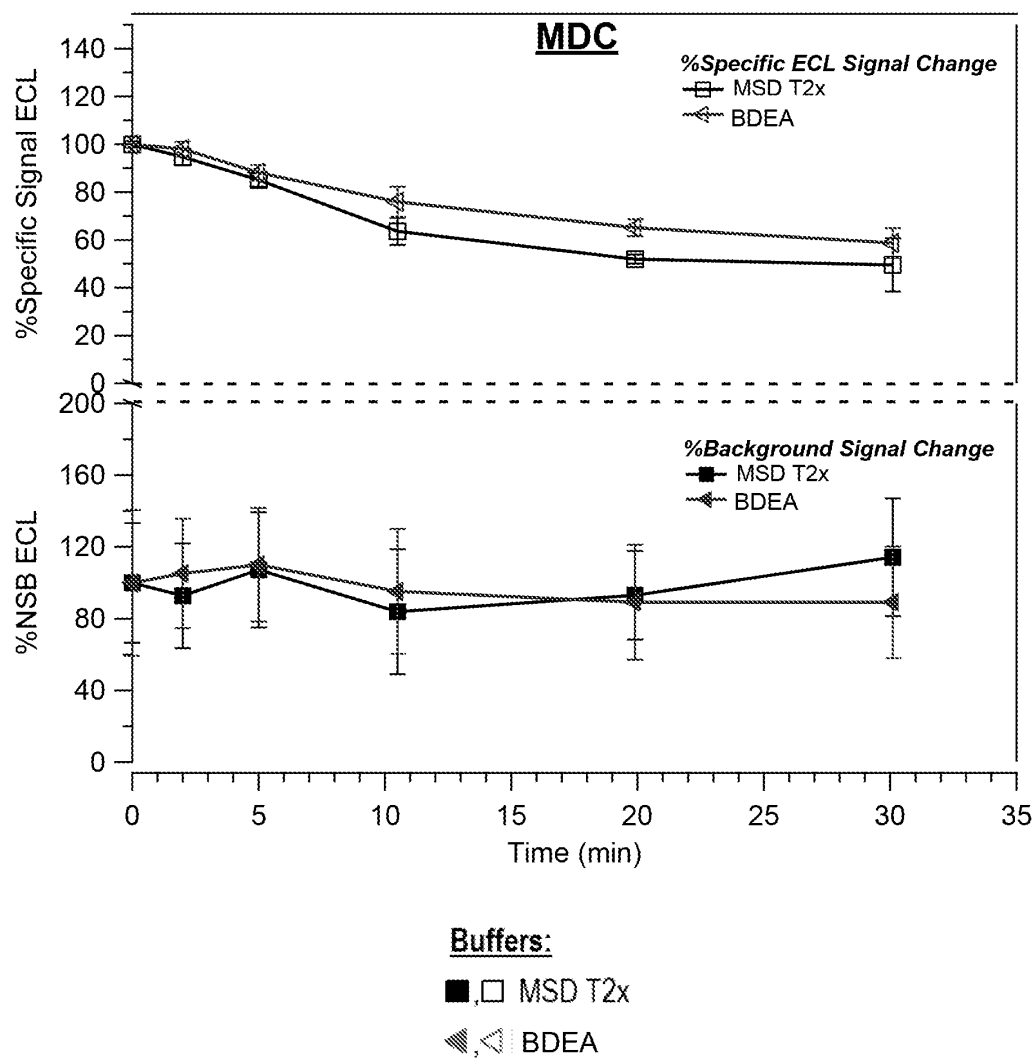
|            |            | Average %ECL loss over 1-4 pg/ml |       |        |       |       |        |
|------------|------------|----------------------------------|-------|--------|-------|-------|--------|
|            |            | MSD T2x                          |       |        | BDEA  |       |        |
| Capture    | Detect     | 0 min                            | 5 min | 10 min | 0 min | 5 min | 10 min |
| 4680-G17-2 | 4681-B70-5 | 0%                               | 38%   | 59%    | 0%    | 20%   | 34%    |

FIG. 9D

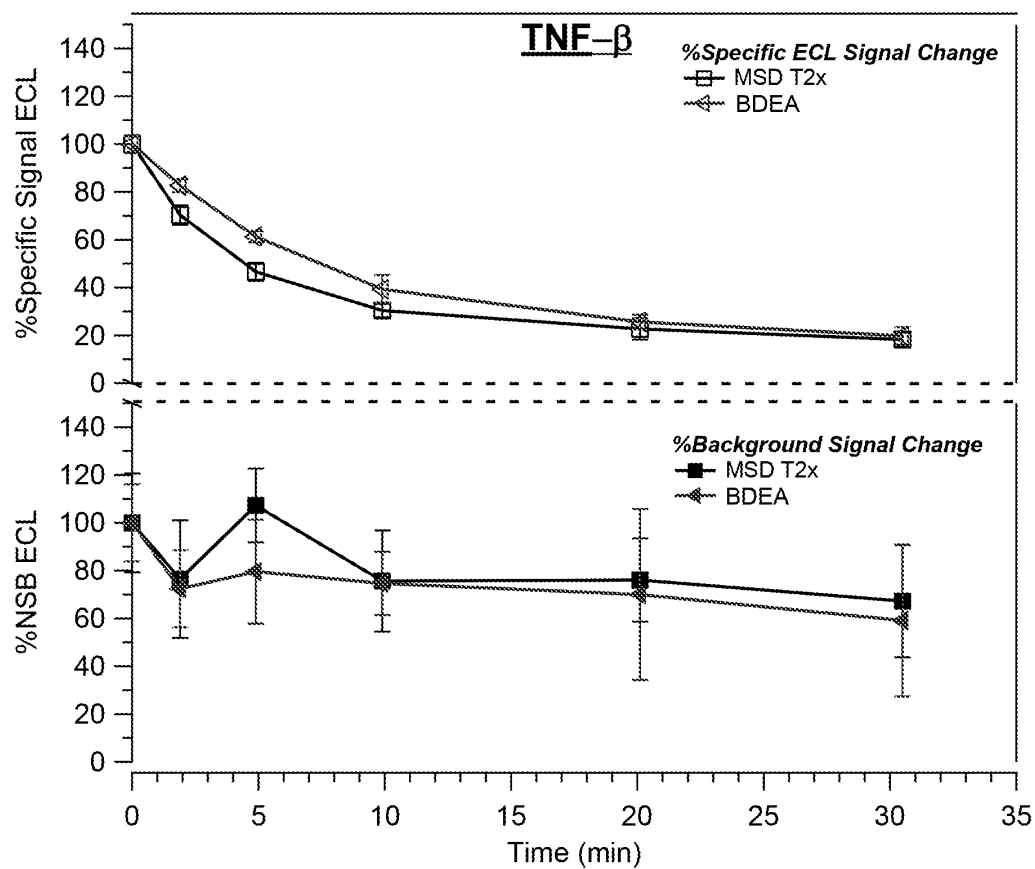
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**FIG. 10A**

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**FIG. 10B**

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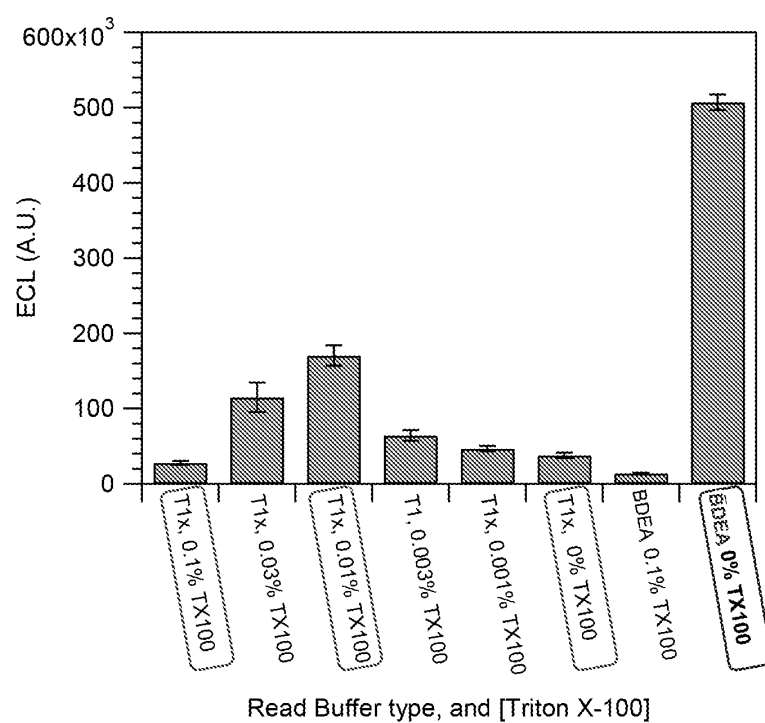
**Buffers:**

■, □ MSD T2x

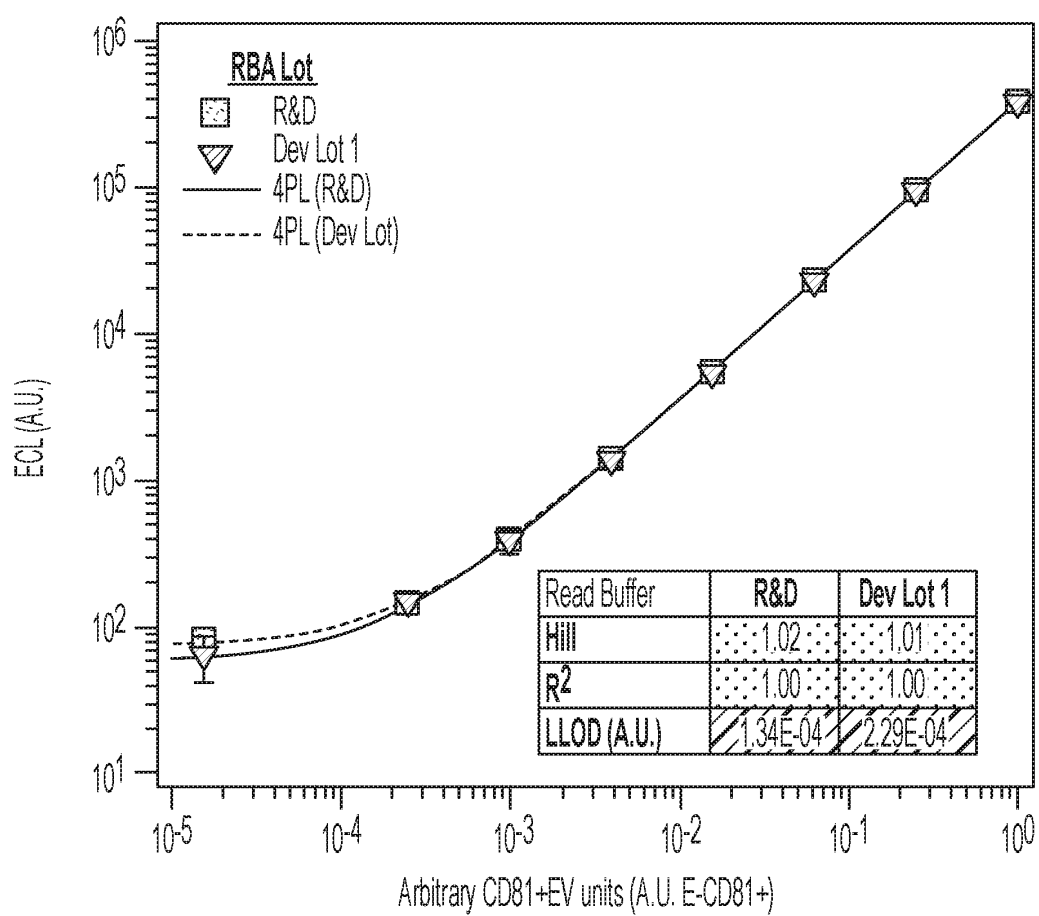
◄, ► BDEA

**FIG. 10C**

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**FIG. 11A**

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**FIG. 11B**