

# RayBio<sup>®</sup> Human Protein Nitration Antibody Array 1

For Simultaneous Detection of the Relative Levels of Nitration  
of 507 Human Proteins

User Manual  
Revised Mar. 22<sup>nd</sup>, 2024

Cat#: AAH-NITRA-G1-4 (4 Sample Kit)  
Cat#: AAH-NITRA-G1-8 (8 Sample Kit)

Please read manual carefully  
before starting experiment





RayBio® Human Protein Nitration Antibody Array 1 Protocol

---

**TABLE OF CONTENTS**

|       |  |    |
|-------|--|----|
| I.    | Introduction.....                        | 2  |
| II.   | Materials Provided.....                  | 3  |
| III.  | Additional Materials Required.....       | 4  |
| IV.   | Reagent Preparation.....                 | 5  |
| V.    | Overview and General Considerations..... | 5  |
|       | A. Preparation of Samples.....           | 5  |
|       | B. Handling Glass Slides.....            | 6  |
|       | C. Incubation.....                       | 6  |
| VI.   | Protocol.....                            | 6  |
|       | A. Dry the Array Slides.....             | 6  |
|       | B. Blocking and Incubation.....          | 7  |
|       | C. Fluorescence Detection.....           | 8  |
| VII.  | Interpretation of Results.....           | 9  |
| VIII. | Troubleshooting Guide.....               | 11 |
| IX.   | Reference List.....                      | 12 |

## I. Introduction

Protein nitration plays an unusually prominent role in cell signaling, development, and growth. The RayBio Human Protein Nitration Antibody Array 1 is a very rapid, convenient, and sensitive assay that can simultaneously detect multiple tyrosine nitration events on proteins. It can also be used to monitor the activation or function of important biological pathways.

RayBiotech is committed to developing a series of nitration antibody arrays. The RayBio Human Protein Nitration Antibody Array 1 is specifically designed for the simultaneous identification of the relative levels of tyrosine nitration in 507 different human proteins in cell lysate. By monitoring changes in protein nitration in your experimental model system, you can verify pathway activation in your cell lines without the need for extensive time and effort required for immunoprecipitation and/or Western Blot analysis.

To use the RayBio Human Protein Nitration Antibody Array 1, treated or untreated cell lysate is added to the antibody array glass slide wells. The antibody array slide wells are then washed, and a biotinylated anti-nitrated-tyrosine antibody is used to detect the nitrated tyrosine residues on target proteins. After incubation with a fluorescent dye-conjugated streptavidin (Cy3 equivalent), the slides can be imaged using a laser scanner, such as the Axon GenePix, in the Cy3 channel.

## II. Materials Provided

Store kit at  $\leq -20$  °C immediately upon arrival. Kit must be used within the 6 months expiration date.

| ITEM                                | COMPONENT  | AAH-NITRA-G1-4    | AAH-NITRA-G1-8     | STORAGE TEMPERATURE AFTER THAWING** |
|-------------------------------------|--|-------------------|--------------------|-------------------------------------|
| 1                                   | RayBio® Glass Slide*                                     | 1                 | 2                  | $\leq -20$ °C                       |
| 2                                   | Blocking Buffer  | 1 bottle (8ml/ea) | 2 bottles (8ml/ea) |                                     |
| 3                                   | Biotinylated Anti-Nitrated-Tyrosine Antibody             | 1 vial            | 2 vials            | 2-8 °C                              |
| 4                                   | Fluorescent Dye-Conjugated Streptavidin (Cy3 equivalent) | 1 vial            | 2 vials            | 2-8 °C                              |
| 5                                   | 20X Wash Buffer I Concentrate                            | 1 bottle (30 ml)  |                    | 2-8 °C                              |
| 6                                   | 20X Wash Buffer II Concentrate                           | 1 bottle (30 ml)  |                    |                                     |
| 7                                   | Wash Buffer III  | 1 bottle (20 ml)  |                    |                                     |
| 8                                   | 2X Cell Lysis Buffer Concentrate                         | 1 bottle (10 ml)  |                    | 2-8 °C                              |
| 9                                   | Protease Inhibitor Cocktail                              | 1 vial            |                    | $\leq -20$ °C                       |
| Other Kit Components: Adhesive film |  |                   |                    |                                     |

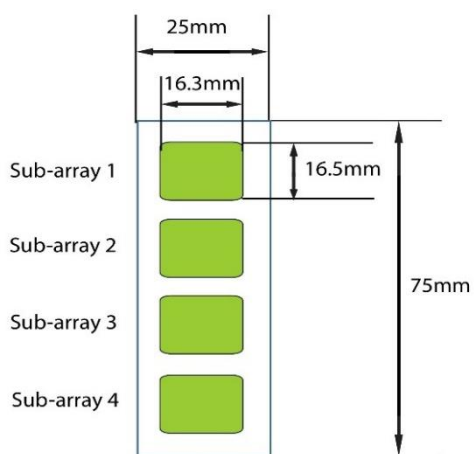
\*Each slide contains 4 identical subarrays

\*\*For up to 3 months (unless stated otherwise) or until expiration date

### III. Additional Materials Required

- Shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Distilled water
- Plastic box
- 50 ml Centrifuge tube
- Isopropanol (2-propanol)

#### Layout of Array Glass Slide



4 printed sub-arrays per glass chip

## IV. Reagent Preparation

1. **Protease Inhibitor Cocktail:** Briefly spin down the Protease Inhibitor Cocktail vial before use. Add 60  $\mu$ l of 1X Cell Lysis Buffer to the vial to prepare a 100X Protease Inhibitor Cocktail Concentrate.
2. **2X Cell Lysis Buffer:** The 2X Cell Lysis Buffer should be diluted 2-fold with deionized or distilled water to prepare a 1X Cell Lysis Buffer solution. Then, add 20  $\mu$ l of the Protease Inhibitor Cocktail Concentrate into 2 ml of the 1X Cell Lysis Buffer to prepare a 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Mix well before use.
3. **20X Wash Buffer I or II:** If the 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 25 ml of the 20X Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1X Wash Buffer.
4. **Wash Buffer III:** Add 15 ml of Wash Buffer III to a tube with 35 ml of isopropanol and mix well. The resulting solution is 30% Wash Buffer III.
5. **Blocking Buffer:** ready to use
6. **Biotinylated anti-Nitrated-Tyrosine Antibodies:** Briefly spin down the Detection Antibody vial before use. Add 90  $\mu$ l of Blocking Buffer to the vial to prepare a Biotinylated Anti-Nitrated-Tyrosine Concentrate. Pipette up and down to mix gently (the Concentrate can be stored at 4 °C for 5 days). Add 90  $\mu$ l of Detection Antibody Concentrate to a tube with 1710  $\mu$ l of Blocking Buffer to prepare a 1X Biotinylated Anti-Nitrated-Tyrosine solution. Mix gently.
7. **Fluorescent Dye-Conjugated Streptavidin (Cy3 equivalent):** Briefly spin down the Fluorescent Dye-Conjugated Streptavidin vial before use. Add 180  $\mu$ l of Blocking Buffer to the vial to prepare a streptavidin concentrate. Pipette up and down to mix gently. Transfer all streptavidin concentrate to a tube with 1.7 ml of Blocking Buffer to prepare a 1X Fluorescent Dye-Conjugated Streptavidin solution. Mix gently.

## V. Overview and General Considerations

### A. Preparation of Samples

#### 1. Cell lysate preparation

For attached cells, remove the supernatant from the cell culture, and wash the cells twice with cold 1X PBS (for cells in suspension, pellet the cells by spinning down at 1500 rpm for 10 min). Make sure to remove any remaining PBS. Then, solubilize the cells at  $2 \times 10^7$  cells/ml in the 1X Cell Lysis Buffer with Protease Inhibitor Cocktail solution. Pipette up and down to resuspend the cells, and rock the lysates gently at 2–8°C for 30 min. Transfer the lysates to microcentrifuge tubes and centrifuge at 14,000 x g for 5 min.

It is recommended that sample protein concentrations be determined using a total protein assay. For incubation with the Nitration Antibody Array 1, use cell lysates at a concentration of 50–1000  $\mu$ g/ml (as a starting point, we recommend using 400  $\mu$ g/ml of cell lysate diluted at least 5-fold with the Blocking Buffer).

Lysates should be used immediately or aliquoted and stored at –80 °C. Thawed lysates should be kept on ice prior to use.

*If you experience high background, you may further dilute your sample.*

## **B. Handling glass slides**

- The microarray slides are very sensitive. Do not touch the array surface with tips, forceps or hands. Hold the slides by the edges only.
- Handle all buffers and slides with powder-free gloves.
- Dry the glass slide completely before the addition of Blocking Buffer.
- Avoid breaking the glass slide when removing the chamber assembly.
- Handle the glass slide in a clean environment.

## **C. Incubation**

- Completely cover the array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- To avoid evaporation, seal the incubation chamber with the provided adhesive film during incubations, particularly when the incubation is more than 2 hours.
- Avoid cross-contamination from overflowing solution to neighboring wells.
- Incubation steps such as step 2 (sample incubation, page 7), or step 7 (Fluorescent Dye-Conjugated Streptavidin incubation, page 7) may be done at 4°C overnight. Please make sure to cover the incubation chamber tightly to prevent evaporation.
- Avoid exposing the array slide to light from step 6 (page 7) onward.

## **VI. Protocol**

### **A. Dry the Glass Slide**

Open the pouch containing the glass slide with frame and take it out. Then let it air dry for 1 hour in a clean environment before use.

**Note:** *Protect the slide from dust or other contaminants.*

### **B. Blocking and Incubation**

1. Add 400 µl of Blocking Buffer to each well and incubate at room temperature with gentle shaking for 30 min to block the slides. Make sure no bubbles are in the wells.
2. Decant the Blocking Buffer from each well (make sure to remove all of the buffer). Add 400 µl of each sample into appropriate wells. Incubate the arrays with sample at room temperature with gentle shaking for 2 hours or at 4°C overnight.

*Note: To aspirate liquid samples or reagents from wells, gently place the pipette tip only in the corners of the well. Do not scrape the pipette tip across the surface of the slide.*

*Note: We recommend using 400  $\mu$ l of cell lysate at a concentration of 50–1000  $\mu$ g/ml (as a starting point, we recommend using 400  $\mu$ g/ml cell lysate). **Dilute the lysate at least 5-fold with the Blocking Buffer. Make sure there are no bubbles in the wells.***

*Note: The amount of sample used depends on the abundance of target proteins. More sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. The optimal sample dilution must be determined empirically by the researcher.*

3. Decant the samples from each well and wash 3 times, 5 min per wash, with 800  $\mu$ l of 1X Wash Buffer I at room temperature with gentle shaking.

*Note: Avoid the solution overflowing into neighboring wells.*

4. Put the glass slide (with frame) into a box with Wash Buffer I (ensure the slide is completely submerged), and wash at room temperature with gentle shaking for 20 min.
5. Decant the Wash Buffer I from each well. Put the glass slide into a box with Wash Buffer II (ensure the slide is completely submerged), and wash 2 times, 5 min per wash, at room temperature with gentle shaking.
6. Remove all of Wash Buffer II from each well. Add 400  $\mu$ l of the 1X Biotin-conjugated Anti-Nitrated-Tyrosine solution to each corresponding well. Incubate at room temperature with gentle shaking for 2 hours.
7. Decant the antibody solution and wash as directed in step 4 three times (wash 3 times, 20 min per wash).
8. Wash as directed in step 5.
9. Remove all of the Wash Buffer II from each well. Add 400  $\mu$ l of the 1X Fluorescent Dye-Conjugated Streptavidin solution to each subarray. Cover the incubation chamber with the adhesive film. Cover the plate with aluminum foil to avoid exposure to light or incubate in a dark room.

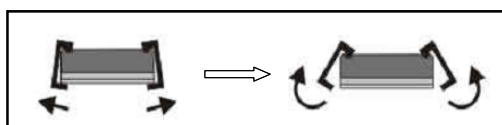
*Note: Avoid exposing the array slide to light from this step forward.*

10. Incubate at room temperature with gentle shaking for 2 hours in the dark.

*Note: Incubation may be done at 4 °C overnight.*

11. Decant the solution and disassemble the glass slide from the incubation frame and chamber. Disassemble the device by pushing clips outward from the side, as shown below. Carefully remove the glass slide from the gasket.

*Note: Be careful not to touch the printed surface of the glass slide, which is on the same side as the barcode.*





12. Gently put the glass slide into a 50 ml centrifuge tube or a plastic box with 40 ml of 1X Wash Buffer I as illustrated below. Gently roll or shake the tube for 5 min. Remove the Wash Buffer I. Repeat 2 more times for a total of 3 washes.



13. Wash the glass slide with 40 ml of Wash Buffer II for 5 min. Repeat one more time for a total of 2 washes.
14. Finally, wash the glass slide with 40 ml of deionized or distilled water.

### **C. Fluorescence Detection**

1. Remove the water droplets from the slide surface by applying suction gently with a pipette tip. Place the glass slide in a laminar flow hood for 20 minutes or until the slide is completely dry. Place the slide under an aluminum foil tent to protect it from light. Make sure the slides are absolutely dry before scanning or storage.
2. Image the slides using a laser scanner, such as the Axon GenePix, using the Cy3 channel.

*Note: We recommend scanning the slides immediately after completing the experiment. Slides can also be stored at  $-20^{\circ}\text{C}$  in the dark for several days. If you do not have a laser scanner, RayBiotech can scan your slide and extract the data for you free of charge.*

*Note: If the background is uneven or too high, put the glass slide into a tube with 40 ml of 30% Wash Buffer III in isopropanol and incubate for 10 min at room temperature (cover the tube with aluminum foil to avoid exposure to light or incubate in a dark room). Dry the slide completely and re-scan the slide.*

## VII. Interpretation of Results

The positive control can be used to identify the orientation of the slide and to normalize the results for comparison of different wells.

The antibody affinity to its target varies significantly between different antibodies. The fluorescence intensity detected on the array with each antibody depends on this affinity; therefore, the signal intensity comparison can only be performed within the same antibody/antigen system and not between different antibodies on the same slide.

### RayBio Human Protein Nitration Antibody Array 1 Array Map

|    |      |      |      |      |      |      |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |      |      |      |      |      |      |
|----|------|------|------|------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|
|    | 1    | 2    | 3    | 4    | 5    | 6    | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25   | 26   | 27   | 28   | 29   | 30   |
| 1  | POS1 | POS1 | POS2 | POS2 | POS3 | POS3 | Neg | Neg | 1   | 1   | 2   | 2   | 3   | 3   | 4   | 4   | 5   | 5   | 6   | 6   | 7   | 7   | 8   | 8   | 9    | 9    | 10   | 10   | 11   | 11   |
| 2  | 12   | 12   | 13   | 13   | 14   | 14   | 15  | 15  | 16  | 16  | 17  | 17  | 18  | 18  | 19  | 19  | 20  | 20  | 21  | 21  | 22  | 22  | 23  | 23  | 24   | 24   | 25   | 25   | 26   | 26   |
| 3  | 27   | 27   | 28   | 28   | 29   | 29   | 30  | 30  | 31  | 31  | 32  | 32  | 33  | 33  | 34  | 34  | 35  | 35  | 36  | 36  | 37  | 37  | 38  | 38  | 39   | 39   | 40   | 40   | 41   | 41   |
| 4  | 42   | 42   | 43   | 43   | 44   | 44   | 45  | 45  | 46  | 46  | 47  | 47  | 48  | 48  | 49  | 49  | 50  | 50  | 51  | 51  | 52  | 52  | 53  | 53  | 54   | 54   | 55   | 55   | 56   | 56   |
| 5  | 57   | 57   | 58   | 58   | 59   | 59   | 60  | 60  | 61  | 61  | 62  | 62  | 63  | 63  | 64  | 64  | 65  | 65  | 66  | 66  | 67  | 67  | 68  | 68  | 69   | 69   | 70   | 70   | 71   | 71   |
| 6  | 72   | 72   | 73   | 73   | 74   | 74   | 75  | 75  | 76  | 76  | 77  | 77  | 78  | 78  | 79  | 79  | 80  | 80  | 81  | 81  | 82  | 82  | 83  | 83  | 84   | 84   | 85   | 85   | 86   | 86   |
| 7  | 87   | 87   | 88   | 88   | 89   | 89   | 90  | 90  | 91  | 91  | 92  | 92  | 93  | 93  | 94  | 94  | 95  | 95  | 96  | 96  | 97  | 97  | 98  | 98  | 99   | 99   | 100  | 100  | 101  | 101  |
| 8  | 102  | 102  | 103  | 103  | 104  | 104  | 105 | 105 | 106 | 106 | 107 | 107 | 108 | 108 | 109 | 109 | 110 | 110 | 111 | 111 | 112 | 112 | 113 | 113 | 114  | 114  | 115  | 115  | 116  | 116  |
| 9  | 117  | 117  | 118  | 118  | 119  | 119  | 120 | 120 | 121 | 121 | 122 | 122 | 123 | 123 | 124 | 124 | 125 | 125 | 126 | 126 | 127 | 127 | 128 | 128 | 129  | 129  | 130  | 130  | 131  | 131  |
| 10 | 132  | 132  | 133  | 133  | 134  | 134  | 135 | 135 | 136 | 136 | 137 | 137 | 138 | 138 | 139 | 139 | 140 | 140 | 141 | 141 | 142 | 142 | 143 | 143 | 144  | 144  | 145  | 145  | 146  | 146  |
| 11 | 147  | 147  | 148  | 148  | 149  | 149  | 150 | 150 | 151 | 151 | 152 | 152 | 153 | 153 | 154 | 154 | 155 | 155 | 156 | 156 | 157 | 157 | 158 | 158 | 159  | 159  | 160  | 160  | 161  | 161  |
| 12 | 162  | 162  | 163  | 163  | 164  | 164  | 165 | 165 | 166 | 166 | 167 | 167 | 168 | 168 | 169 | 169 | 170 | 170 | 171 | 171 | 172 | 172 | 173 | 173 | 174  | 174  | 175  | 175  | 176  | 176  |
| 13 | 177  | 177  | 178  | 178  | 179  | 179  | 180 | 180 | 181 | 181 | 182 | 182 | 183 | 183 | 184 | 184 | 185 | 185 | 186 | 186 | 187 | 187 | 188 | 188 | 189  | 189  | 190  | 190  | 191  | 191  |
| 14 | 192  | 192  | 193  | 193  | 194  | 194  | 195 | 195 | 196 | 196 | 197 | 197 | 198 | 198 | 199 | 199 | 200 | 200 | 201 | 201 | 202 | 202 | 203 | 203 | 204  | 204  | 205  | 205  | 206  | 206  |
| 15 | 207  | 207  | 208  | 208  | 209  | 209  | 210 | 210 | 211 | 211 | 212 | 212 | 213 | 213 | 214 | 214 | 215 | 215 | 216 | 216 | 217 | 217 | 218 | 218 | 219  | 219  | 220  | 220  | 221  | 221  |
| 16 | 222  | 222  | 223  | 223  | 224  | 224  | 225 | 225 | 226 | 226 | 227 | 227 | 228 | 228 | 229 | 229 | 230 | 230 | 231 | 231 | 232 | 232 | 233 | 233 | 234  | 234  | 235  | 235  | 236  | 236  |
| 17 | 237  | 237  | 238  | 238  | 239  | 239  | 240 | 240 | 241 | 241 | 242 | 242 | 243 | 243 | 244 | 244 | 245 | 245 | 246 | 246 | 247 | 247 | 248 | 248 | 249  | 249  | 250  | 250  | 251  | 251  |
| 18 | 252  | 252  | 253  | 253  | 254  | 254  | 255 | 255 | 256 | 256 | 257 | 257 | 258 | 258 | 259 | 259 | 260 | 260 | 261 | 261 | 262 | 262 | 263 | 263 | 264  | 264  | 265  | 265  | 266  | 266  |
| 19 | 267  | 267  | 268  | 268  | 269  | 269  | 270 | 270 | 271 | 271 | 272 | 272 | 273 | 273 | 274 | 274 | 275 | 275 | 276 | 276 | 277 | 277 | 278 | 278 | 279  | 279  | 280  | 280  | 281  | 281  |
| 20 | POS1 | POS1 | POS2 | POS2 | POS3 | POS3 | Neg | Neg | 282 | 282 | 283 | 283 | 284 | 284 | 285 | 285 | 286 | 286 | 287 | 287 | 288 | 288 | 289 | 289 | 290  | 290  | 291  | 291  | 292  | 292  |
| 21 | 293  | 293  | 294  | 294  | 295  | 295  | 296 | 296 | 297 | 297 | 298 | 298 | 299 | 299 | 300 | 300 | 301 | 301 | 302 | 302 | 303 | 303 | 304 | 304 | 305  | 305  | 306  | 306  | 307  | 307  |
| 22 | 308  | 308  | 309  | 309  | 310  | 310  | 311 | 311 | 312 | 312 | 313 | 313 | 314 | 314 | 315 | 315 | 316 | 316 | 317 | 317 | 318 | 318 | 319 | 319 | 320  | 320  | 321  | 321  | 322  | 322  |
| 23 | 323  | 323  | 324  | 324  | 325  | 325  | 326 | 326 | 327 | 327 | 328 | 328 | 329 | 329 | 330 | 330 | 331 | 331 | 332 | 332 | 333 | 333 | 334 | 334 | 335  | 335  | 336  | 336  | 337  | 337  |
| 24 | 338  | 338  | 339  | 339  | 340  | 340  | 341 | 341 | 342 | 342 | 343 | 343 | 344 | 344 | 345 | 345 | 346 | 346 | 347 | 347 | 348 | 348 | 349 | 349 | 350  | 350  | 351  | 351  | 352  | 352  |
| 25 | 353  | 353  | 354  | 354  | 355  | 355  | 356 | 356 | 357 | 357 | 358 | 358 | 359 | 359 | 360 | 360 | 361 | 361 | 362 | 362 | 363 | 363 | 364 | 364 | 365  | 365  | 366  | 366  | 367  | 367  |
| 26 | 368  | 368  | 369  | 369  | 370  | 370  | 371 | 371 | 372 | 372 | 373 | 373 | 374 | 374 | 375 | 375 | 376 | 376 | 377 | 377 | 378 | 378 | 379 | 379 | 380  | 380  | 381  | 381  | 382  | 382  |
| 27 | 383  | 383  | 384  | 384  | 385  | 385  | 386 | 386 | 387 | 387 | 388 | 388 | 389 | 389 | 390 | 390 | 391 | 391 | 392 | 392 | 393 | 393 | 394 | 394 | 395  | 395  | 396  | 396  | 397  | 397  |
| 28 | 398  | 398  | 399  | 399  | 400  | 400  | 401 | 401 | 402 | 402 | 403 | 403 | 404 | 404 | 405 | 405 | 406 | 406 | 407 | 407 | 408 | 408 | 409 | 409 | 410  | 410  | 411  | 411  | 412  | 412  |
| 29 | 413  | 413  | 414  | 414  | 415  | 415  | 416 | 416 | 417 | 417 | 418 | 418 | 419 | 419 | 420 | 420 | 421 | 421 | 422 | 422 | 423 | 423 | 424 | 424 | 425  | 425  | 426  | 426  | 427  | 427  |
| 30 | 428  | 428  | 429  | 429  | 430  | 430  | 431 | 431 | 432 | 432 | 433 | 433 | 434 | 434 | 435 | 435 | 436 | 436 | 437 | 437 | 438 | 438 | 439 | 439 | 440  | 440  | 441  | 441  | 442  | 442  |
| 31 | 443  | 443  | 444  | 444  | 445  | 445  | 446 | 446 | 447 | 447 | 448 | 448 | 449 | 449 | 450 | 450 | 451 | 451 | 452 | 452 | 453 | 453 | 454 | 454 | 455  | 455  | 456  | 456  | 457  | 457  |
| 32 | 458  | 458  | 459  | 459  | 460  | 460  | 461 | 461 | 462 | 462 | 463 | 463 | 464 | 464 | 465 | 465 | 466 | 466 | 467 | 467 | 468 | 468 | 469 | 469 | 470  | 470  | 471  | 471  | 472  | 472  |
| 33 | 473  | 473  | 474  | 474  | 475  | 475  | 476 | 476 | 477 | 477 | 478 | 478 | 479 | 479 | 480 | 480 | 481 | 481 | 482 | 482 | 483 | 483 | 484 | 484 | 485  | 485  | 486  | 486  | 487  | 487  |
| 34 | 488  | 488  | 489  | 489  | 490  | 490  | 491 | 491 | 492 | 492 | 493 | 493 | 494 | 494 | 495 | 495 | 496 | 496 | 497 | 497 | 498 | 498 | 499 | 499 | 500  | 500  | 501  | 501  | 502  | 502  |
| 35 | 503  | 503  | 504  | 504  | 505  | 505  | 506 | 506 | 507 | 507 | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | POS3 | POS3 | POS2 | POS2 | POS1 | POS1 |

# RayBio Human Protein Nitration Antibody Array 1 Target List

| Number | Name            | Number | Name           | Number | Name               | Number | Name            | Number | Name         | Number | Name            | Number | Name               |
|--------|-----------------|--------|----------------|--------|--------------------|--------|-----------------|--------|--------------|--------|-----------------|--------|--------------------|
| 1      | GCKine          | 74     | F3             | 147    | FGF-19             | 220    | IGFBP-4         | 293    | IL-22 BP     | 366    | MMP-20          | 439    | Shh-N              |
| 2      | Activin A       | 75     | CRIM 1         | 148    | FGF-20             | 221    | IGFBP-6         | 294    | IL-22 R      | 367    | MMP-24          | 440    | SPARC              |
| 3      | Activin B       | 76     | Cripto-1       | 149    | FGF-21             | 222    | IGFBP-rp1       | 295    | IL-23        | 368    | MMP-25          | 441    | Spinesin           |
| 4      | Activin C       | 77     | CRTH-2         | 150    | FGF-23             | 223    | IGF-1           | 296    | IL-23 R      | 369    | MSPa            | 442    | TACI               |
| 5      | Activin RIA     | 78     | Cryptic        | 151    | FLRG               | 224    | IGF-1 R         | 297    | IL-24        | 370    | Musk            | 443    | Tarc               |
| 6      | Activin RIB     | 79     | Csk            | 152    | Flt-3 Ligand       | 225    | IGF-II          | 298    | IL-26        | 371    | NAP-2           | 444    | TCCR               |
| 7      | EYA2            | 80     | CTACK          | 153    | Follistatin        | 226    | IGF-II R        | 299    | IL-27        | 372    | NCAM-1          | 445    | TECK               |
| 8      | Activin RIIA    | 81     | CTGF           | 154    | Follistatin-like 1 | 227    | IL-1 alpha      | 300    | IL-28A       | 373    | Neuritin        | 446    | TFPI               |
| 9      | Adiponectin     | 82     | CTLA-4         | 155    | Fractalkine        | 228    | IL-1 beta       | 301    | IL-29        | 374    | NeuroD1         | 447    | TGF-alpha          |
| 10     | AgRP            | 83     | CV-2           | 156    | Frizzled-1         | 229    | IL-1 F5         | 302    | IL-31        | 375    | Neuropilin-2    | 448    | TGF-beta 1         |
| 11     | ALCAM           | 84     | CXCL14         | 157    | Frizzled-3         | 230    | IL-1 F6         | 303    | IL-31 RA     | 376    | Neurturin       | 449    | TGF-beta 2         |
| 12     | Angiogenin      | 85     | CXCL16         | 158    | Frizzled-4         | 231    | IL-1 F7         | 304    | BACE-1       | 377    | NGF R           | 450    | TGF-beta 3         |
| 13     | Angiopoietin-1  | 86     | CXCR1          | 159    | Frizzled-5         | 232    | IL-1 F8         | 305    | FACX         | 378    | Nidogen-1       | 451    | ATP2B1             |
| 14     | Angiopoietin-2  | 87     | CXCR2          | 160    | Frizzled-6         | 233    | IL-1 F9         | 306    | Insulin      | 379    | NOV             | 452    | TGF-beta RI        |
| 15     | Angiopoietin-4  | 88     | CXCR3          | 161    | Frizzled-7         | 234    | IL-1 F10        | 307    | Insulin R    | 380    | NrCam           | 453    | TGF-beta RII       |
| 16     | ANGPTL1         | 89     | CXCR4          | 162    | Galectin-3         | 235    | IL-1 R3         | 308    | Insulysin    | 381    | GGF2            | 454    | Grb2               |
| 17     | ANGPTL2         | 90     | CXCR5          | 163    | GASP-1             | 236    | IL-1 R4         | 309    | IP-10        | 382    | NRG2            | 455    | TGF-beta RIII      |
| 18     | ANGPTL7         | 91     | CXCR6          | 164    | GASP-2             | 237    | IL-1 R6         | 310    | I-TAC        | 383    | NRG3            | 456    | Thrombopoietin     |
| 19     | Angiostatin     | 92     | D6             | 165    | GCP-2              | 238    | IL-1 R8         | 311    | Kininostatin | 384    | NT-3            | 457    | Thyroid Peroxidase |
| 20     | APJ             | 93     | DAN            | 166    | GCSF               | 239    | IL-1 R9         | 312    | Kremen-1     | 385    | NT-4            | 458    | Thrombospondin-1   |
| 21     | APRIL           | 94     | DANCE          | 167    | G-CSF R            | 240    | IL-1 ra         | 313    | Kremen-2     | 386    | Orexin A        | 459    | Thrombospondin-2   |
| 22     | Amphiregulin    | 95     | DcR3           | 168    | GDF1               | 241    | IL-1 RI         | 314    | LTBP1        | 387    | Orexin B        | 460    | Thrombospondin-4   |
| 23     | Artemin         | 96     | Decorin        | 169    | GDF3               | 242    | IL-1 RII        | 315    | LBP          | 388    | OSM             | 461    | Thymopoietin       |
| 24     | Axl             | 97     | Dkk-1          | 170    | GDF5               | 243    | IL-2            | 316    | Lck          | 389    | Osteoactivin    | 462    | Tie-1              |
| 25     | B7-1            | 98     | Dkk-3          | 171    | GDF8               | 244    | IL-2 R alpha    | 317    | LECT2        | 390    | Osteocrin       | 463    | Tie-2              |
| 26     | BAFF R          | 99     | Dkk-4          | 172    | GDF9               | 245    | IL-2 R beta     | 318    | Lefty-A      | 391    | Osteoprotegerin | 464    | TIMP-1             |
| 27     | BCMA            | 100    | DR3            | 173    | GDF11              | 246    | IL-2 R gamma    | 319    | Leptin       | 392    | OX40 Ligand     | 465    | TIMP-2             |
| 28     | BD-1            | 101    | DR6            | 174    | GDF-15             | 247    | IL-3            | 320    | Leptin R     | 393    | PARC            | 466    | TIMP-3             |
| 29     | BDNF            | 102    | Dtk            | 175    | GDNF               | 248    | IL-3 R alpha    | 321    | LFA-1 alpha  | 394    | PD-ECGF         | 467    | TIMP-4             |
| 30     | beta-Catenin    | 103    | EDA-A2         | 176    | GFR alpha-1        | 249    | IL-4            | 322    | LIF          | 395    | PDGF R alpha    | 468    | DEFA5              |
| 31     | Bax             | 104    | EDAR           | 177    | GFR alpha-2        | 250    | IL-4 R          | 323    | LIF R alpha  | 396    | PDGF R beta     | 469    | TLR1               |
| 32     | beta-NGF        | 105    | EDG-1          | 178    | GFR alpha-3        | 251    | IL-5            | 324    | LIGHT        | 397    | PDGF-AA         | 470    | TLR2               |
| 33     | BIK             | 106    | EGF            | 179    | GFR alpha-4        | 252    | IL-5 R alpha    | 325    | Lipocalin-1  | 398    | PDGF-AB         | 471    | TLR3               |
| 34     | BLC             | 107    | EGF R          | 180    | GITR               | 253    | IL-6            | 326    | Lipocalin-2  | 399    | PDGF-BB         | 472    | TLR4               |
| 35     | BMP-2           | 108    | EG-VEGF        | 181    | GITR Ligand        | 254    | IL-6 R          | 327    | LRP-1        | 400    | PDGF-C          | 473    | TMEFF1             |
| 36     | BMP-3           | 109    | EMAP-II        | 182    | CBR1               | 255    | IL-7            | 328    | LRP-6        | 401    | PDGF-D          | 474    | TMEFF2             |
| 37     | BMP-3b          | 110    | ENA-78         | 183    | Glut1              | 256    | IL-7 R alpha    | 329    | L-Selectin   | 402    | PECAM-1         | 475    | TNF-alpha          |
| 38     | BMP-4           | 111    | Endocan        | 184    | Glut2              | 257    | IL-8            | 330    | Lymphotactin | 403    | Pentraxin3      | 476    | TNF-beta           |
| 39     | BMP-5           | 112    | Endoglin       | 185    | Glut3              | 258    | IL-9            | 331    | LTB          | 404    | Perserpin       | 477    | TNF RI             |
| 40     | BMP-6           | 113    | Endostatin     | 186    | Glut5              | 259    | IL-10           | 332    | LTBR         | 405    | PF4             | 478    | TNF RII            |
| 41     | BMP-7           | 114    | Endothelin     | 187    | Glypican 3         | 260    | IL-10 R alpha   | 333    | MAC-1        | 406    | PIGF            | 479    | TRADD              |
| 42     | BMP-8           | 115    | EN-RAGE        | 188    | Glypican 5         | 261    | IL-10 R beta    | 334    | MCP-1        | 407    | PLUNC           | 480    | TRAIL              |
| 43     | BMP-15          | 116    | Eotaxin        | 189    | GM-CSF             | 262    | IL-11           | 335    | MCP-2        | 408    | Pref-1          | 481    | TRAIL R1           |
| 44     | BMPRI-A         | 117    | Eotaxin-2      | 190    | GM-CSF R alpha     | 263    | IL-12 p40       | 336    | MCP-3        | 409    | Progranulin     | 482    | TRAIL R2           |
| 45     | BMPRI-B         | 118    | Eotaxin-3      | 191    | Granzyme A         | 264    | IL-12 p70       | 337    | MCP-4        | 410    | Prolactin       | 483    | TRAIL R3           |
| 46     | BMPRII          | 119    | Epregrulin     | 192    | GREMLIN            | 265    | IL-12 R beta 1  | 338    | M-CSF        | 411    | P-selectin      | 484    | TRAIL R4           |
| 47     | BTC             | 120    | ErbB2          | 193    | GRO                | 266    | IL-12 R beta 2  | 339    | M-CSF R      | 412    | RAGE            | 485    | TRANCE             |
| 48     | Cardiotrophin-1 | 121    | ErbB3          | 194    | GRO-a              | 267    | IL-13           | 340    | MDC          | 413    | RANK            | 486    | TREM-1             |
| 49     | CCL14           | 122    | ErbB4          | 195    | GH                 | 268    | IL-13 R alpha 1 | 341    | MFG-E8       | 414    | RANTES          | 487    | TROY               |
| 50     | CCL28           | 123    | Erythropoietin | 196    | GHR                | 269    | IL-13 R alpha 2 | 342    | MFRP         | 415    | RELM beta       | 488    | TSG-6              |
| 51     | CCR1            | 124    | E-Selectin     | 197    | HB-EGF             | 270    | IL-15           | 343    | MICA         | 416    | RELT            | 489    | TSLP R             |
| 52     | CCR2            | 125    | FADD           | 198    | HCC-4              | 271    | IL-15 R alpha   | 344    | MIF          | 417    | ROBO4           | 490    | TWEAK              |
| 53     | CCR3            | 126    | FAM3B          | 199    | HCR                | 272    | IL-16           | 345    | MIG          | 418    | S100 A8/A9      | 491    | TWEAK R            |
| 54     | CCR4            | 127    | Fas            | 200    | Hepassocin         | 273    | IL-17           | 346    | MIP-1a       | 419    | S100A10         | 492    | Ubiquitin+1        |
| 55     | CCR5            | 128    | Fas Ligand     | 201    | GLO-1              | 274    | IL-17B          | 347    | MIP-1b       | 420    | SAA             | 493    | uPA                |
| 56     | CCR6            | 129    | FGF Basic      | 202    | HGF                | 275    | IL-17B R        | 348    | MIP-1d       | 421    | SCF             | 494    | uPAR               |
| 57     | CCR7            | 130    | FGF-BP         | 203    | HGFR               | 276    | IL-17C          | 349    | MIP 2        | 422    | SCF R           | 495    | Vasorin            |
| 58     | CCR8            | 131    | FGF R3         | 204    | HRG-alpha          | 277    | IL-17D          | 350    | MIP-3 alpha  | 423    | SDF-1           | 496    | VCAM-1             |
| 59     | CCR9            | 132    | FGF R4         | 205    | HRG-beta 1         | 278    | IL-17E          | 351    | MIP-3 beta   | 424    | sFRP-1          | 497    | VE-Cadherin        |
| 60     | CD14            | 133    | FGF R5         | 206    | HVEM               | 279    | IL-17F          | 352    | MMP-1        | 425    | sFRP-3          | 498    | VEGF               |
| 61     | CD27            | 134    | FGF-4          | 207    | I-309              | 280    | IL-17R          | 353    | MMP-2        | 426    | sFRP-4          | 499    | VEGF R2            |
| 62     | CD30            | 135    | FGF-5          | 208    | ICAM-1             | 281    | IL-17RC         | 354    | MMP-3        | 427    | sgp130          | 500    | VEGF R3            |
| 63     | CD30 Ligand     | 136    | FGF-6          | 209    | ICAM-2             | 282    | IL-17RD         | 355    | MMP-7        | 428    | SIGIRR          | 501    | VEGF-B             |
| 64     | CD40            | 137    | FGF-7          | 210    | ICAM-3             | 283    | IL-18 BPa       | 356    | MMP-8        | 429    | Siglec-5        | 502    | VEGF-C             |
| 65     | CD40 Ligand     | 138    | FGF-8          | 211    | ICAM-5             | 284    | IL-18 R alpha   | 357    | MMP-9        | 430    | Siglec-9        | 503    | VEGF-D             |
| 66     | CD 163          | 139    | FGF-9          | 212    | IFN-alpha/beta R1  | 285    | IL-18 R beta    | 358    | MMP-10       | 431    | SLPI            | 504    | VEGI               |
| 67     | Cerberus 1      | 140    | FGF-10         | 213    | IFN-alpha/beta R2  | 286    | IL-19           | 359    | MMP-11       | 432    | Smad 1          | 505    | WIF-1              |
| 68     | Chem R23        | 141    | FGF-11         | 214    | IFN-beta           | 287    | IL-20           | 360    | MMP-12       | 433    | Smad 4          | 506    | WISP-1             |
| 69     | Chordin-Like 1  | 142    | FGF-12         | 215    | IFN-gamma          | 288    | IL-20 R alpha   | 361    | MMP-13       | 434    | Smad 5          | 507    | XEDAR              |
| 70     | Chordin-Like 2  | 143    | FGF-13 1B      | 216    | IFN-gamma R1       | 289    | IL-20 R beta    | 362    | MMP-14       | 435    | Smad 7          |        |                    |
| 71     | CLC             | 144    | FGF-16         | 217    | IGFBP-1            | 290    | IL-21           | 363    | MMP-15       | 436    | Smad 8          |        |                    |
| 72     | CNTF            | 145    | FGF-17         | 218    | IGFBP-2            | 291    | IL-21 R         | 364    | MMP-16       | 437    | Prdx6           |        |                    |
| 73     | CNTF R alpha    | 146    | FGF-18         | 219    | IGFBP-3            | 292    | IL-22           | 365    | MMP-19       | 438    | Soggy-1         |        |                    |

## VIII. Troubleshooting Guide

| <b>Problem</b>  | <b>Cause</b>                                    | <b>Recommendation</b>  |
|-----------------|---|--|
| Weak Signal     | Inadequate detection                            | Increase laser power and PMT parameters  |
|                 | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation                                    |
|                 | Short incubation time                           | Ensure sufficient incubation time and change sample incubation step to overnight |
|                 | Too low protein concentration in sample         | Reduce sample dilution or concentrate sample                                     |
|                 | Improper storage of kit                         | Store kit at suggested temperature. Don't freeze/thaw the slide.                 |
| Uneven signal   | Bubble formed during incubation                 | Avoid bubble formation during incubation   |
|                 | Arrays are not completely covered by reagent    | Completely cover arrays with solution  |
|                 | Reagent evaporation                             | Cover the incubation chamber with adhesive film during incubation                |
| High background | Excess of biotinylated protein                  | Make sure to use the correct amount of protein                                   |
|                 | Excess of streptavidin                          | Make sure to use the correct amount of streptavidin                              |
|                 | Inadequate detection                            | Check laser power and PMT parameters   |
|                 | Inadequate wash                                 | Increase the volume of wash buffer and incubation time                           |

## IX. Reference List

Profiling receptor tyrosine kinase activation by using Ab microarrays. Nielsen UB, Cardone MH, Sinskey AJ, MacBeath G, and Sorger PK. **PNAS**. 2003;100(16):9330-9335.

A Prototype Antibody Microarray Platform to Monitor Changes in Protein Tyrosine Phosphorylation. Gembitsky DS, Lawlor K, Jacovina A, Yaneva M, and Tempst P. **Mol Cell Proteomics**. 2004;3:1102-1118.

Analysis of receptor signaling pathways by mass spectrometry: Identification of Vav-2 as a substrate of the epidermal and platelet derived growth factor receptors. Pandey A, Podtelejnikov AV, Blagoev B, Bustelo XR, Mann M, and Lodish HF. **PNAS**. 2000; 97(1);179-184.

Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 overexpression and prostaglandin e(2) secretion in patients with breast cancer". Pockaj BA, Basu GD. **Annal Surg Oncol**. 2004;3:327-344.

Cytokine Antibody Arrays: A Promising Tool to Identify Molecular Targets for Drug Discovery. Huang RP. **Comb Chem High Throughput Screen**. 2003;6:79-99.

Connexin suppresses human glioblastoma cell growth by down-regulation of monocyte chemotactic protein 1, as discovered using protein array technology. Huang R, Lin Y, Wang CC, J et al. **Cancer Res**. 2002;62:2806-2812.

Profiling of cytokine expression by biotin-labeled-based protein arrays. Lin Y, Huang R, Chen L-P, et al. **Proteomics**. 2003, 3: 1750-1757.

A novel method for high- throughput protein profiling from conditioned media and patient's sera. Huang RP, Huang R, Fan Y, and Lin Y. **Ana. Biochem**. 2001;294(1):55-62.

RayBio® Cytokine Antibody Arrays are patent-pending technology developed by RayBiotech.

This product is intended *for research only* and is not to be used for clinical diagnosis. Our products may not be resold, modified for resale, or used to manufacture commercial products without written approval by RayBiotech Life, Inc.

Under no circumstances shall RayBiotech be liable for any damages arising out of the use of the materials.

Products are guaranteed for 6 months from the date of purchase when handled and stored properly. In the event of any defect in quality or merchantability, RayBiotech's liability to buyer for any claim relating to products shall be limited to replacement or refund of the purchase price.

RayBio® is a registered trademark of RayBiotech Life, Inc.

**This product is for research use only.**



**©2012 RayBiotech Life, Inc.**