

**human XIAP ASSAY LAYOUT SHEET**  
for use with Assay Designs' Catalog No. 900-124

• **TABLE FOR MAKING STANDARDS 1-6\***

\* Reconstitute the 5,000 pg/vial human XIAP Standard with 500  $\mu$ L Assay Buffer 21. Let sit 5 minutes, then mix gently. **Subsequent standards should be diluted in polypropylene test tubes and used within 20 minutes of preparation.**

| Std. | Assay Buffer 21 Vol. ( $\mu$ L) | Standard Vol. Added ( $\mu$ L) | human XIAP Conc. (pg/mL) |
|------|---------------------------------|--------------------------------|--------------------------|
| 1    | 500                             | Lyoph.                         | 10,000                   |
| 2    | 250                             | 250, Std. 1                    | 5,000                    |
| 3    | 250                             | 250, Std. 2                    | 2,500                    |
| 4    | 250                             | 250, Std. 3                    | 1,250                    |
| 5    | 250                             | 250, Std. 4                    | 625                      |
| 6    | 250                             | 250, Std. 5                    | 312.5                    |

• **ASSAY PROTOCOL FLOW CHART**

| Well ID:                               | Blank<br>A1, B1 | S 0<br>C1-D1 | Standards<br>E1-H2 | Samples<br>A3-H12 |
|--|-----------------|--------------|--------------------|-------------------|
| Assay Buffer 21                        | ---             | 100 $\mu$ L  | ---                | ---               |
| Std. or Sample                         | ---             | ---          | 100 $\mu$ L        | 100 $\mu$ L       |
| Seal plate and tap gently to mix       | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Incub. 1 hour @ RT, shaking and sealed | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400 $\mu$ L            | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Antibody                               | ---             | 100 $\mu$ L  | 100 $\mu$ L        | 100 $\mu$ L       |
| Incub. 1 hour @ RT, shaking & sealed   | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400 $\mu$ L            | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Conjugate                              | ---             | 100 $\mu$ L  | 100 $\mu$ L        | 100 $\mu$ L       |
| Incub. 30 min. @ RT, shaking & sealed  | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Asp. & Wash 4 x 400 $\mu$ L            | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| TMB Substrate                          | 100 $\mu$ L     | 100 $\mu$ L  | 100 $\mu$ L        | 100 $\mu$ L       |
| Incub. 30 min. @ RT, shaking & sealed  | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |
| Stop Solution 2 (1N HCl)               | 100 $\mu$ L     | 100 $\mu$ L  | 100 $\mu$ L        | 100 $\mu$ L       |
| Read OD at 450 nm                      | ⇒⇒⇒⇒            | ⇒⇒⇒⇒         | ⇒⇒⇒⇒               | ⇒⇒⇒⇒              |

**human XIAP PLATE LAYOUT:**

|          |          |    |    |    |    |    |    |    |     |     |     |
|----------|----------|----|----|----|----|----|----|----|-----|-----|-----|
| A1 Blank | A2 Std 3 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | A10 | A11 | A12 |
| B1 Blank | B2 Std 3 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 |
| C1 S0    | C2 Std 4 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| D1 S0    | D2 Std 4 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
| E1 Std 1 | E2 Std 5 | E3 | E4 | E5 | E6 | E7 | E8 | E9 | E10 | E11 | E12 |
| F1 Std 1 | F2 Std 5 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 | F12 |
| G1 Std 2 | G2 Std 6 | G3 | G4 | G5 | G6 | G7 | G8 | G9 | G10 | G11 | G12 |
| H1 Std 2 | H2 Std 6 | H3 | H4 | H5 | H6 | H7 | H8 | H9 | H10 | H11 | H12 |

Kit Lot No. \_\_\_\_\_ Exp. Date \_\_\_\_\_ Date \_\_\_\_\_ Tech. \_\_\_\_\_

1<sup>st</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

3<sup>rd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

2<sup>nd</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_

4<sup>th</sup> Incub.: Start Time \_\_\_\_\_ Temp. \_\_\_\_\_  
 End Time \_\_\_\_\_ Temp. \_\_\_\_\_