# Assays Using COS Cells Transfected with AR

| Reference | Kemppainen and Wilson (1996) | Kemppainen et al. (1992) |
|---|---|---|
| **Characteristics of Cells** | | |
| *Cell line* | COS-1 | COS-7 |
| *Cell source* | monkey kidney | monkey kidney |
| *Source of receptor* | pCMVhAR | pCMVhAR |
| *AR source* | human | human |
| *Transfection of AR* | Transient | Transient |
| *Whole cells/cell homogenate/cytosol* | whole cells | whole cells |
| **Preparation of Cells for Assay** | | |
| *Serum source* | Fetal calf serum | Fetal calf serum |
| *Serum stripping method* | n.p. | n.p. |
| *Residual androgen in serum* | n.p. | n.p. |
| *No. of treated cells/No. of cells homogenized* | $2x10^5$ cells/well | $1x10^5$ cells/well |
| *Treatment vessel used* | 12-well plates | 24-well culture dishes |
| *Preparation of cell homogenate* | n.a. | n.a. |
| *volume* | n.a. | n.a. |
| *buffer* | n.a. | n.a. |
| *method* | n.a. | n.a. |
| *time; temperature* | n.a. | n.a. |
| *Centrifugation of homogenate* | n.a. | n.a. |
| *Protein concentration of cytosol* | n.a. | n.a. |
| *Storage* | n.a. | n.a. |
| *Final protein concentration* | n.a. | n.a. |
| *Separation of bound hormone* | Phosphate buffer saline wash | Phosphate buffer saline wash |
| **Competitive Binding Assay** | | |
| *Reference ligand* | R1881 | R1881 |
| *Volume and concentration of reference ligand* | 5 nM | 5 nM |
| *Specific activity of labelled reference ligand* | 80 Ci/mmol | 80 Ci/mmol |
| *Volume and concentration of cold ligand* | 100-fold molar excess | 100-fold molar excess |
| *Final concentration of reference ligand* | 5 nM | 5 nM |
| *Volume of competing ligand* | n.p. | n.p. |
| *Concentration range of competing ligand* | 5-500 nM | 5-500 nM |
| *Volume of cytosol* | n.a. | n.a. |
| *Volume of buffer* | n.p. | n.p. |
| *Type of buffer used* | n.p. | n.p. |
| *Replicates* | n.p. | n.p. |
| *Time of incubation* | 2 hr | 2 hr |
| *Temperature of incubation* | 37° C | 37° C |

# Assays Using COS Cells Transfected with AR

| Reference | Kemppainen and Wilson (1996) | Kemppainen et al. (1992) |
|---|---|---|
| **Separation of ligand** | | |
| *Volume and type of slurry* | n.p. | n.p. |
| *Buffer for slurry* | n.p. | n.p. |
| *Incubation time and temp* | n.p. | n.p. |
| *Time of vortexing* | n.p. | n.p. |
| *Centrifugation speed* | n.p. | n.p. |
| *Centrifugation time and temp* | n.p. | n.p. |
| *Resuspension volume and buffer for pellet* | n.p. | n.p. |
| *No. of washes* | 2 | 2 |
| *Extraction of label* | n.p. | n.p. |
| *Incubation time and temperature* | n.p. | n.p. |
| *Vortexing during incubation time* | n.p. | n.p. |
| *Centrifugation time and temperature* | n.p. | n.p. |
| *Volume added for reading* | n.p. | n.p. |
| *Volume of fluor* | n.p. | n.p. |
| *Type of fluor* | n.p. | n.p. |
| *Instrumentation* | n.p. | n.p. |
| *Measurement* | n.p. | n.p. |
| *Blank without competitor* | n.p. | n.p. |
| *Reading of blank* | n.p. | n.p. |
| *Blank subtracted?* | n.p. | n.p. |
| *Range of standard curve of reference ligand* | n.p. | n.p. |
| *Nonspecific binding measured?* | n.p. | n.p. |
| *Subtraction of nonspecific binding* | n.p. | n.p. |
| **Data calculations** | | |
| *Data plotted as* | % [$^3$H]-R1881 vs. Unlabeled ligand (μM) | % [$^3$H]-R1881 vs. Unlabeled hormone (nM) |
| *Data calculated* | n.p. | n.p. |
| *Calculation of RBA* | Estimated from competitive binding graph | Estimated from competitive binding graph |
| **Test substances** | | |
| *Solvent used* | n.p. | n.p. |
| *No. of samples/ dose* | n.p. | n.p. |
| *No. of times assay repeated* | n.p. | n.p. |
| Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity | | |

# Assays Using COS Cells Transfected with AR

| Reference | Kemppainen et al. (1999) | Lambright et al. (2000) |
|---|---|---|
| **Characteristics of Cells** | | |
| *Cell line* | COS (otherwise undefined) | COS (otherwise undefined) |
| *Cell source* | monkey kidney | monkey kidney |
| *Source of receptor* | pCMVhAR | pCMVhAR |
| *AR source* | human | human |
| *Transfection of AR* | Transient | Transient |
| *Whole cells/cell homogenate/cytosol* | whole cells | whole cells |
| **Preparation of Cells for Assay** | | |
| *Serum source* | Fetal calf serum | n.p. |
| *Serum stripping method* | n.p. | n.p. |
| *Residual androgen in serum* | n.p. | n.p. |
| *No. of treated cells/No. of cells homogenized* | $3.5x10^5$ cells/well | n.p. |
| *Treatment vessel used* | 6-well plates | n.p. |
| *Preparation of cell homogenate* | n.a. | n.a. |
| *volume* | n.a. | n.a. |
| *buffer* | n.a. | n.a. |
| *method* | n.a. | n.a. |
| *time; temperature* | n.a. | n.a. |
| *Centrifugation of homogenate* | n.a. | n.a. |
| *Protein concentration of cytosol* | n.a. | n.a. |
| *Storage* | n.a. | n.a. |
| *Final protein concentration* | n.a. | n.a. |
| *Separation of bound hormone* | Phosphate buffer saline wash | n.p. |
| **Competitive Binding Assay** | | |
| *Reference ligand* | R1881 | R1881 |
| *Volume and concentration of reference ligand* | 5 nM | 5 nM |
| *Specific activity of labelled reference ligand* | n.p. | n.p. |
| *Volume and concentration of cold ligand* | 10,000-fold molar excess | n.p. |
| *Final concentration of reference ligand* | 5 nM | n.p. |
| *Volume of competing ligand* | n.p. | n.p. |
| *Concentration range of competing ligand* | n.p. | n.p. |
| *Volume of cytosol* | n.a. | n.p. |
| *Volume of buffer* | n.p. | n.p. |
| *Type of buffer used* | n.p. | n.p. |
| *Replicates* | n.p. | n.p. |
| *Time of incubation* | 2 hr | 2 hr |
| *Temperature of incubation* | 37° C | 37° C |

# Assays Using COS Cells Transfected with AR

| Reference | Kemppainen et al. (1999) | Lambright et al. (2000) |
|---|---|---|
| **Separation of ligand** | | |
| *Volume and type of slurry* | n.p. | n.p. |
| *Buffer for slurry* | n.p. | n.p. |
| *Incubation time and temp* | n.p. | n.p. |
| *Time of vortexing* | n.p. | n.p. |
| *Centrifugation speed* | n.p. | n.p. |
| *Centrifugation time and temp* | n.p. | n.p. |
| *Resuspension volume and buffer for pellet* | n.p. | n.p. |
| *No. of washes* | 1 | n.p. |
| *Extraction of label* | n.p. | n.p. |
| *Incubation time and temperature* | n.p. | n.p. |
| *Vortexing during incubation time* | n.p. | n.p. |
| *Centrifugation time and temperature* | n.p. | n.p. |
| *Volume added for reading* | n.p. | n.p. |
| *Volume of fluor* | n.p. | n.p. |
| *Type of fluor* | n.p. | n.p. |
| *Instrumentation* | n.p. | n.p. |
| *Measurement* | n.p. | n.p. |
| *Blank without competitor* | n.p. | n.p. |
| *Reading of blank* | n.p. | n.p. |
| *Blank subtracted?* | n.p. | n.p. |
| *Range of standard curve of reference ligand* | n.p. | n.p. |
| *Nonspecific binding measured?* | n.p. | n.p. |
| *Subtraction of nonspecific binding* | n.p. | n.p. |
| **Data calculations** | | |
| *Data plotted as* | Scatchard plots | n.p. |
| *Data calculated* | Inhibition constant ($K_i$) and $IC_{50}$ | n.p. |
| *Calculation of RBA* | From $IC_{50}$ values | n.p. |
| **Test substances** | | |
| *Solvent used* | n.p. | n.p. |
| *No. of samples/ dose* | n.p. | n.p. |
| *No. of times assay repeated* | n.p. | n.p. |
| Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity | | |

# Assays Using COS Cells Transfected with AR

| Reference | Takeo and Yamashita (2000) | Tilley et al. (1989) |
|---|---|---|
| **Characteristics of Cells** | | |
| *Cell line* | COS-1 | COS-1 |
| *Cell source* | monkey kidney | monkey kidney |
| *Source of receptor* | rtARα expression vector | pCMVhAR |
| *AR source* | rainbow trout | human |
| *Transfection of AR* | Transient | Transient |
| *Whole cells/cell homogenate/cytosol* | cytosol | cell homogenate |
| **Preparation of Cells for Assay** | | |
| *Serum source* | n.p. | n.p. |
| *Serum stripping method* | n.p. | n.p. |
| *Residual androgen in serum* | n.p. | n.p. |
| *No. of treated cells/No. of cells homogenized* | n.p. | n.p. |
| *Treatment vessel used* | n.p. | n.p. |
| *Preparation of cell homogenate* | n.p. | n.p. |
| *volume* | n.p. | 2-3:1 |
| *buffer* | n.p. | Tris-EDTA, pH 7.2 |
| *method* | n.p. | aspiration thru 25 Ga needle |
| *time; temperature* | n.p. | n.p. |
| *Centrifugation of homogenate* | n.p. | 250,000xg, 30 min |
| *Protein concentration of cytosol* | n.p. | 1.5 mg/ml |
| *Storage* | n.p. | n.p. |
| *Final protein concentration* | n.p. | 0.3 mg |
| *Separation of bound hormone* | n.p. | Dextran-charcoal |
| **Competitive Binding Assay** | | |
| *Reference ligand* | Mibolerone | 5α-Dihydrotestosterone |
| *Volume and concentration of reference ligand* | 1 nM | 3 nM |
| *Specific activity of labelled reference ligand* | n.p. | n.p. |
| *Volume and concentration of cold ligand* | n.p. | n.p. |
| *Final concentration of reference ligand* | n.p. | 3 nM |
| *Volume of competing ligand* | n.p. | n.p. |
| *Concentration range of competing ligand* | 1-1000 nM | 3 - 300 nM |
| *Volume of cytosol* | n.a. | 0.2 ml |
| *Volume of buffer* | n.p. | n.p. |
| *Type of buffer used* | n.p. | TEGM, pH 7.2 |
| *Replicates* | n.p. | n.p. |
| *Time of incubation* | 5 hr | 5 hr |
| *Temperature of incubation* | 4° C | 4° C |

# Assays Using COS Cells Transfected with AR

| Reference | Takeo and Yamashita (2000) | Tilley et al. (1989) |
|---|---|---|
| **Separation of ligand** | | |
| *Volume and type of slurry* | dextran-charcoal, 50 µl | dextran-charcoal, 50 µl |
| *Buffer for slurry* | Tris, pH 7.2 | Tris, pH 7.2 |
| *Incubation time and temp* | 5 min, 0° C | 5 min, 0° C |
| *Time of vortexing* | n.p. | n.p. |
| *Centrifugation speed* | 2000xg | 2000xg |
| *Centrifugation time and temp* | 10 min, 0° C | 10 min, 0° C |
| *Resuspension volume and buffer for pellet* | 5 ml | 5 ml |
| *No. of washes* | 1 | 1 |
| *Extraction of label* | n.p. | n.p. |
| *Incubation time and temperature* | n.p. | n.p. |
| *Vortexing during incubation time* | n.p. | n.p. |
| *Centrifugation time and temperature* | n.p. | 2000xg, 10 min |
| *Volume added for reading* | n.p. | n.p. |
| *Volume of fluor* | 5 ml | 5 ml |
| *Type of fluor* | n.p. | n.p. |
| *Instrumentation* | n.p. | n.p. |
| *Measurement* | n.p. | n.p. |
| *Blank without competitor* | n.p. | n.p. |
| *Reading of blank* | n.p. | n.p. |
| *Blank subtracted?* | n.p. | n.p. |
| *Range of standard curve of reference ligand* | n.p. | n.p. |
| *Nonspecific binding measured?* | n.p. | n.p. |
| *Subtraction of nonspecific binding* | n.p. | n.p. |
| **Data calculations** | | |
| *Data plotted as* | Graphpad prism software | % DHT binding |
| *Data calculated* | n.p. | n.p. |
| *Calculation of RBA* | Estimated from competitive binding graph | Estimated from competitive binding graph |
| **Test substances** | | |
| *Solvent used* | n.p. | n.p. |
| *No. of samples/ dose* | n.p. | n.p. |
| *No. of times assay repeated* | n.p. | n.p. |
| Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity | | |

# Assays Using COS Cells Transfected with AR

| Reference | Wong et al. (1995) |
|---|---|
| **Characteristics of Cells** | |
| *Cell line* | COS-1 |
| *Cell source* | monkey kidney |
| *Source of receptor* | pCMVhAR |
| *AR source* | human |
| *Transfection of AR* | Transient |
| *Whole cells/cell homogenate/cytosol* | whole cells |
| **Preparation of Cells for Assay** | |
| *Serum source* | Fetal calf serum |
| *Serum stripping method* | n.p. |
| *Residual androgen in serum* | n.p. |
| *No. of treated cells/No. of cells homogenized* | $1x10^5$ cells/well |
| *Treatment vessel used* | 12-well plates |
| *Preparation of cell homogenate* | n.a. |
| *volume* | n.a. |
| *buffer* | n.a. |
| *method* | n.a. |
| *time; temperature* | n.a. |
| *Centrifugation of homogenate* | n.a. |
| *Protein concentration of cytosol* | n.a. |
| *Storage* | n.a. |
| *Final protein concentration* | n.a. |
| *Separation of bound hormone* | Phosphate buffer saline wash |
| **Competitive Binding Assay** | |
| *Reference ligand* | R1881 |
| *Volume and concentration of reference ligand* | 5 nM |
| *Specific activity of labelled reference ligand* | 85.5 Ci/mmol |
| *Volume and concentration of cold ligand* | 100-fold molar excess |
| *Final concentration of reference ligand* | 5 nM |
| *Volume of competing ligand* | n.p. |
| *Concentration range of competing ligand* | .005 -50 µM |
| *Volume of cytosol* | n.a. |
| *Volume of buffer* | n.p. |
| *Type of buffer used* | n.p. |
| *Replicates* | 3 |
| *Time of incubation* | 2 hr |
| *Temperature of incubation* | 37° C |

# Assays Using COS Cells Transfected with AR

| Reference | Wong et al. (1995) |
|---|---|
| **Separation of ligand** | |
| *Volume and type of slurry* | n.p. |
| *Buffer for slurry* | n.p. |
| *Incubation time and temp* | n.p. |
| *Time of vortexing* | n.p. |
| *Centrifugation speed* | n.p. |
| *Centrifugation time and temp* | n.p. |
| *Resuspension volume and buffer for pellet* | n.p. |
| *No. of washes* | 2 |
| *Extraction of label* | n.p. |
| *Incubation time and temperature* | n.p. |
| *Vortexing during incubation time* | n.p. |
| *Centrifugation time and temperature* | n.p. |
| *Volume added for reading* | n.p. |
| *Volume of fluor* | n.p. |
| *Type of fluor* | n.p. |
| *Instrumentation* | n.p. |
| *Measurement* | n.p. |
| *Blank without competitor* | n.p. |
| *Reading of blank* | n.p. |
| *Blank subtracted?* | n.p. |
| *Range of standard curve of reference ligand* | n.p. |
| *Nonspecific binding measured?* | n.p. |
| *Subtraction of nonspecific binding* | n.p. |
| **Data calculations** | |
| *Data plotted as* | % [$^3$H]-R1881vs. Unlabeled ligand (μM) |
| *Data calculated* | n.p. |
| *Calculation of RBA* | Estimated from competitive binding graph |
| **Test substances** | |
| *Solvent used* | n.p. |
| *No. of samples/ dose* | n.p. |
| *No. of times assay repeated* | 3 |

Abbreviations: n.a. = not applicable; n.p. = not provided; RBA = relative binding affinity